

# ANNALS OF BOTANY

EDITED BY

V. H. BLACKMAN, Sc.D., F.R.S.

314

RESEARCH INSTITUTE OF PLANT PHYSIOLOGY, IMPERIAL  
COLLEGE OF SCIENCE AND TECHNOLOGY, LONDON

ASSISTED BY

A. J. EAMES, Ph.D.

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ITHACA, N.Y., U.S.A.

F. W. OLIVER, M.A., D.Sc., F.R.S.

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AND OTHER BOTANISTS

NEW SERIES. VOLUME VI

With ten Plates and four hundred and seventy Figures

1942

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## Physiological Studies in Plant Nutrition

### XII. Carbohydrate Changes in the several Organs of the Barley Plant during Growth, with especial Reference to the Development and Ripening of the Ear

BY

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AND

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With nine Figures in the Text

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## INTRODUCTION

THE immediate origin of the starch of cereal grains has been the subject of discussion for some time. At first it was assumed that in the absence of any starch-storing organ the supply for the grain must originate from carbon compounds elaborated in the leaves and thence translocated directly to the grain. Déhérain and Dupont (1901), however, pointed out that in wheat, which is without starch reserves in the leaves or elsewhere, starch accumulates rapidly in the grain during the last weeks before harvest, although at this stage the leaves are senescent. They sought for an alternative assimilating surface to the leaves, and were able to show in a qualitative manner that the

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parts of the stem remaining green in the late stages of growth were capable of assimilation. They placed pieces of stem in a closed vessel containing air enriched with carbon dioxide and noted the disappearance of the carbon dioxide in the light. Similar experiments with spikes did not afford evidence of assimilation by these organs at the same stage (but see below, p. 3). Finally, they demonstrated that removal of spikes from plants in the field resulted in an accumulation of sugars in the stem within twenty-four hours and concluded that these sugars would normally have travelled to the ears. Although it was known at the time that cereal stems contained both sucrose and fructosans (Kühnemann, 1875; Schulze, 1894), information as to the amounts and seasonal variation of these sugars was lacking, and Dèhèrain and Dupont do not suggest the possibility of stored sugar forming a source of supply for the grain. Stalfelt (1935) measured the assimilation rate of stems and sheaths of oats and gives the ratio of assimilating powers of leaf:stem:sheath as 100:40:19; and he points out that the assimilating surface of stem and sheath is greater than that of the leaves. His figures are not applicable to field conditions but confirm the earlier observation that a considerable amount of assimilation occurs in organs other than the leaves.

From 1922 onwards Colin and Belval published a number of papers dealing with seasonal changes in sugar concentration in cereals; a bibliography containing full references to these papers is included in a review dealing with fructosans (Archbold, 1940). They found that the soluble sugars of the stems, and especially fructosans, increased in concentration up to flowering and subsequently fell. They suggest that the sugars formed in the leaves are stored in the stems until the ears begin to 'shoot', when they migrate to the ear and are finally converted to starch. The high concentration of fructosan in the immature ear and its subsequent disappearance led them to regard fructosan as a possible precursor of starch; but Belval (1924) notes that the increase in the ratio of starch to sugar in the ear is far too large to be accounted for mainly by the conversion to starch of the fructosan in the immature ear. Nevertheless, the view that stored sugars of the stems and ears form an important source of carbohydrate for the ear has been widely held.

The seasonal drift of sugar concentration established by Colin and Belval has been amply confirmed in wheat by Barnell (1936, 1938), in barley by Archbold (1938), and in oats by Phillips et al. (1939). When, however, the results of Barnell's and Archbold's analyses of wheat and barley were examined on the basis of quantity per plant it became evident that much of the starch in the grain must arise direct from new assimilates in the late stages of growth. Barnell states that 'the total loss of sucrose in the whole plant from the time of maximum sucrose content to the end of the season accounts for less than half the amount of starch formed in the ears', while Archbold found that the ear dry weight increased steadily for four weeks after ear emergence without any depletion of dry matter (or loss of sugar) from the rest of the plant. Barnell's samples were collected at rather long intervals, and Arch-



bold's collections did not continue right up to harvest, so that neither set of data provides conclusive evidence as to the amount of stored sugar translocated to the ear, although both suggest that it may account for only a small part of the starch accumulating there. A further source of uncertainty lies in the fact that interchanges with the roots are not considered in any of the above experiments, and only Barnell includes sugars in a combination which may resist the mild reagents required to hydrolyse sucrose and fructosan.

In addition to assimilation by the stem it has now become clear that the ear itself contributes carbohydrate by assimilation. Thus Harlan and Anthony (1920) found that removal of awns of barley as they appeared resulted in a 25 per cent. decrease in the grain yield, the difference in final weight being largely due to starch. Watson and Norman (1939), in a paper published during the progress of the present work, state as a result of shading experiments that 19 to 28 per cent. of the final dry weight of the ear is accounted for by autonomous assimilation, and quote similar results for wheat obtained by Boonstra and by Smith.

The available evidence clearly suggests that as the leaves, stem, and ears are successively developed, all supply photosynthate for conversion to starch in the ear; and in addition excess sugar stored before the ear shoots may be reutilized after translocation to the ear. The purpose of the present work has been to ascertain the proportionate contributions to ear development of each organ by assimilation, and of any retranslocated material. The data set forth here deal particularly with the part played by stored sugar; data relating to the contributions of the stems and ears to the total assimilate will be presented in a subsequent paper.

The study relating to stored sugar has been carried out during two seasons, 1938 and 1940. In 1938 attention was especially directed to the analysis of roots and to determining the distribution and variation in time of combined glucose other than that occurring in sucrose. In 1940 the growing conditions were altered as to the number of plants per pot and the amount of nitrogen added, in order to induce high concentrations of sugar in the stem. Determinations made during two-thirds, approximately, of the growth cycle (the very early stages being omitted) included dry weight, residual dry weight,<sup>1</sup> total sugar (after hydrolysis with N/5 H<sub>2</sub>SO<sub>4</sub>), and its constituent fructose and glucose, and the additional glucose produced by hydrolysis with N acid. Leaves, flag-leaf sheaths, stems (including sheaths other than that of the flag leaf), and ears were separately analysed. From the data estimates of the losses of stored sugar and other materials which may be translocated upwards have been made and compared with the simultaneous gains in weight of the developing ears.

<sup>1</sup> 'Residual dry weight' throughout this paper denotes the material remaining after extraction of tissue with 95 per cent. alcohol, followed by cold water; it is not the same as the 'residual dry weight' of Maskell (1928), where the term is used to mean total dry weight less sugar.



## COLLECTION OF MATERIAL

The barley variety used was a pure line Plumage Archer. The seeds were sterilized in formalin before being planted in 10-in. glazed pots in soil known to be deficient in nitrogen. The pots were placed in the open in double rows on stands facing north and south to make shading effects as uniform as possible, and were covered in wet weather by 'windolite' shades to avoid flooding. When necessary they were watered with tap-water. At the time of full emergence of the first leaf the plants were thinned to the required number, and a dressing of nitrate given. Duplicate samples were collected at intervals varying from two days to a week, always at 10 a.m. The pots to be used at each collection were assigned at random at the start. In 1938 one pot (three plants) constituted each sample. In 1940 there were six plants per pot, four receiving shading and defoliation treatments with which the present paper is not concerned. Five untreated plants, one from each of five pots, made up each sample. The times of sowing and collecting, &c., are shown in Table I.

TABLE I

*Data of Barley Samples collected during the Seasons 1938, 1940*

	1938.	1940.
Sowing date . . . . .	May 13	April 29
No. seeds per pot . . . . .	9	18
No. of plants after thinning . . . . .	3	6
Date of first collection . . . . .	June 13	June 28
„ harvest . . . . .	Sept. 27	Sept. 12
NaNO <sub>3</sub> added per pot . . . . .	6 gm.	2 gm.
No. plants per sample . . . . .	3*	5

\* On June 13, 20, 27, and July 4 owing to the small size of the plants the numbers per sample were 30, 15, 9, and 6 respectively.

The plants were cut at the junction of root and stem and dealt with as described by Archbold (1938), aliquots being preserved in alcohol for the sugar determinations and others used for the dry-weight determinations. For collection of roots the soil was turned out into a vat of water and the roots gently shaken. The water was poured off through a sieve and fresh water added and the process repeated until the roots were free from soil. The roots were gently squeezed to remove excess water and weighed. Aliquots of 10 gm. were then taken for dry weight and 10–20 gm. for sugar determinations.

## METHODS OF ANALYSIS

The methods of analysis used are those described in detail by Archbold (1938) and Vanderplank (1936). Briefly, dry weights were determined by drying well-spread-out material at 70–75° C., using a water-pump to draw a current of air through the oven. Sugars were estimated in uncleared solutions by the Harding and Downs (1932) copper reagent. Sucrose, fructosan, and hexoses were included in one solution by the addition of the cold-water

extract of the residue from alcoholic extraction to the evaporated alcoholic extract. The question of clarification of barley extracts has been discussed by Archbold (1938). Woody stems were macerated prior to water extraction by the wet grinding mill devised by Russell (1938). In the case of roots the sugar concentration was always low and it was necessary to evaporate further the combined alcohol and water extracts under reduced pressure to obtain a suitable concentration for analysis. In all 1940 samples the solutions were again evaporated after cold-water treatment, so that sufficient water could be used to ensure complete extraction of the smaller samples and a suitable final volume for sugar estimations obtained. The size of the aliquots varied from 2 to 10 gm. fresh weight. The residual material after extraction was dried at 100° C. and weighed. It consists mainly of cellulose complexes and alcohol denatured proteins, together with some mineral constituents. It is this fraction which is termed residual dry weight.

Archbold (1938) has shown that in extracts of barley leaves and stems the amounts of fermentable reducing substances (using a copper reagent) show little seasonal variation, so that if required a suitable correction of the sugar values obtained in uncleared solutions can be made by the use of an average figure. The average values found from determinations throughout the growth cycle were 1.00 per cent. of the dry weight for leaves, 0.15 per cent. for stems, and 0.16 per cent. for ears. Corresponding values for roots have now been determined and are given in Table II.

TABLE II

*Reducing Power of Barley Roots before and after Fermentation. (Extracts of 10–20 gm. of root concentrated to 50 ml. and fermented 16 hours at 37° C. with baker's yeast)*

Apparent sugar (per cent. of dry weight).

1938.	Before hydrolysis.		After hydrolysis (N/5 acid).	
	Before fermentation.	After fermentation.	Before fermentation.	After fermentation.
July 20 . .	0.080	0.047	0.860	0.087
„ 22 . .	0.141	0.050	1.500	0.066
„ 25 . .	0.093	0.060	2.416	0.100
„ 27 . .	0.504	0.056	—	—
„ 29 . .	0.587	0.106	—	—
Aug. 2 . .	0.125	0.030	1.350	0.040
„ 4 . .	0.300	0.085	—	—
„ 6 . .	0.530	0.107	4.538	0.123
„ 16 . .	0.294	0.063	1.473	0.072
„ 29 . .	0.184	0.043	0.895	0.086
Sept. 27 . .	0.075	0.065	0.495	0.010
Mean . .	0.265	0.065	1.815	0.073

Unfermentable reducing power (as glucose):

as percentage of reducing sugar, 24; of total sugar, 4.

Amos and Woo ! (1939) state that in extracts of the aerial parts of *Lolium subulatum* Vis. there was an increase in the unfermentable reducing material

after hydrolysis with N/5 acid, and therefore consider it necessary to carry out fermentations before and after hydrolysis in order to obtain correct sugar values. Under the conditions used for barley extracts no consistent differences were found in this fraction after hydrolysis with N/5 acid, although there was an increase when stronger acid was employed (see p. 7). The values obtained before and after hydrolysis with N/5 acid are given in Table III.

TABLE III

*Unfermentable Reducing Power of Barley Extracts before and after Hydrolysis with N/5 Sulphuric Acid*

Percentage of apparent glucose in the fresh weight.			
	1938.	Before hydrolysis.	After hydrolysis.
Leaves . . .	Aug. 4	0.162	0.186
" . . .	" 16	0.198	0.207
Sheaths . . .	" 3	0.065	0.065
" . . .	" 22	0.164	0.164
Stems . . .	" 3	0.045	0.052
" . . .	" 4	0.097	0.058
Ears . . .	" 3	0.065	0.065
" . . .	" 4	0.114	0.091

Vanderplank (1936) has pointed out that potassium ferricyanide (used by Amos and Wood) exerts a stronger oxidizing action on non-sugar substances in plant extracts than does alkaline copper, which may account for the differences in the results obtained with *Lolium* and with barley. A further difference in technique lies in the time allowed for the fermentation of hexoses to reach completion. Amos and Wood allowed 20 minutes, compared with 2 to 16 hours in these experiments according to the activity of the particular samples of yeast. None of our samples fermented fructose in less than 100 minutes and many took much longer, although glucose was completely fermented in 10 to 15 minutes. The production of fructose from fructosans on hydrolysis would, of course, increase any error due to incomplete fermentation of fructose.

For the estimation of glucose in glycosidic combination hydrolysis with N acid is generally employed, and it is well known that in most plant extracts such treatment results in the liberation of a greater quantity of glucose than hydrolysis by N/5 or weaker acid or by invertase. N acid destroys fructose fairly readily, so that either the glycosides must be separated from sucrose and fructosans or the amount of fructose destroyed determined. Barnell (1936) attempted to separate the glycoside fraction in wheat by precipitation of solutions, prepared from alcoholic extracts, with basic lead acetate, and then hydrolysing the lead precipitate for an hour. Phillis and Mason (1933) hydrolysed extracts of the cotton plant for 3 hours with N HCl, at the end of which time fructose was completely destroyed. They state that the glucose yield from glycoside (polyglucoside) reached its maximum in about an hour. As it is usually difficult to define conditions under which polysaccharides and



glucosides are completely precipitated by basic lead acetate without removal of simple sugars, the barley extracts have been hydrolysed with N sulphuric acid without clarification and the course of hydrolysis determined with respect to both glucose and fructose. Hydrolysis curves for these two sugars are shown in Fig. 1. Glucose reached its maximum value in about three hours,

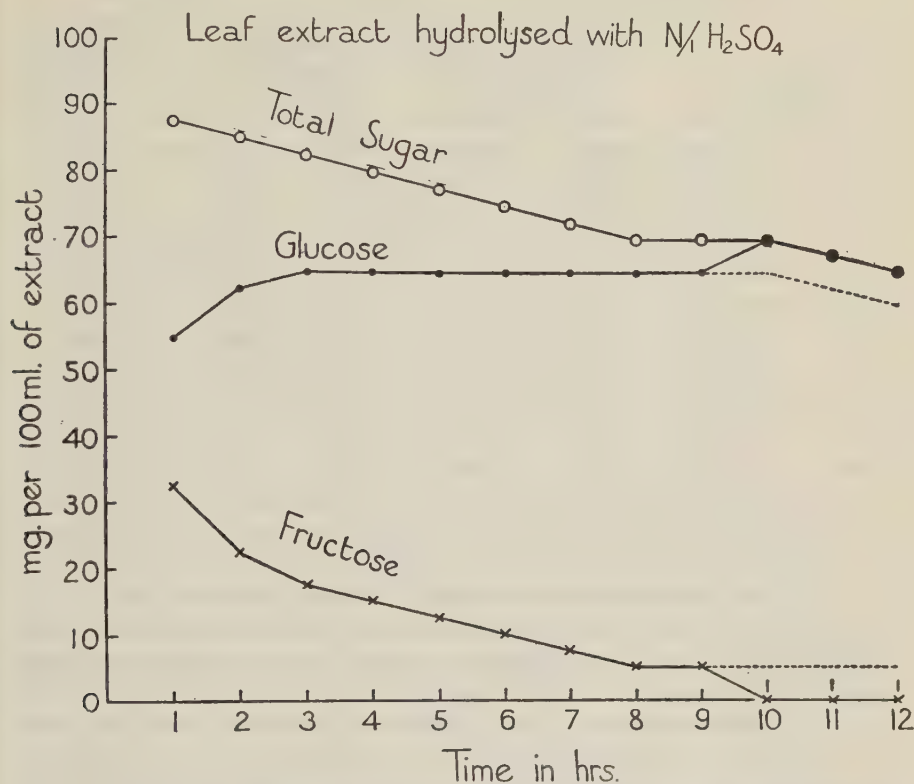


FIG. 1. The production of fructose and glucose by the action of  $N\ H_2SO_4$  on an extract of barley leaves. The broken line in the fructose curve shows the maximum amount of fructose which could escape detection by the analytical method used, and in the glucose curve the corresponding values for that sugar.

and the barley glycoside is therefore more resistant to hydrolysis ( $N\ H_2SO_4$ ) than that of cotton ( $N\ HCl$ ). Fructose was continuously destroyed, but complete destruction was not approached in 3 hours with sulphuric acid as the hydrolytic agent. For routine determinations hydrolysis was continued for 4 hours to ensure liberation of all the glucose, and both glucose and fructose estimated in the hydrolysate. Eighty-five per cent. of the additional reducing power thus obtained and estimated as glucose was fermentable, and there was a clear increase in unfermentable reducing substance. Allowance should be made for this increase in making estimates of glycoside glucose in

barley extracts. The values obtained for a series of extracts of barley leaves are shown in Table IV.

TABLE IV

*Estimates of Glycoside-glucose (per cent. fresh weight) in Extracts of Barley Leaves gathered at different Stages of the Growth Cycle*

1 Hydrolysed with N/5 H <sub>2</sub> SO <sub>4</sub> .		2 Hydrolysed with N H <sub>2</sub> SO <sub>4</sub> .		Unfermentable sugar (as glucose) in:		Increase in fermentable sugar (glycoside, glucose).
Fructose.	Glucose.	Fructose.	Glucose.	1.	2.	
0·88	0·69	0·31	1·44	0·23	0·25	0·73
0·94	0·64	0·35	1·07	0·08	0·13	0·38
0·97	0·84	0·38	1·56	0·20	0·35	0·57
1·66	1·15	0·66	1·84	0·28	0·40	0·57
1·25	0·67	0·42	1·21	0·15	0·15	0·54
1·66	1·09	0·60	1·65	0·26	0·30	0·52
1·67	1·50	0·92	1·62	0·10	0·17	0·05
2·15	1·40	0·83	1·92	0·16	0·20	0·48
2·19	1·73	1·25	1·83	0·15	0·22	0·03
2·20	1·65	1·08	1·92	0·24	0·38	0·13
2·25	1·56	0·84	2·22	0·20	0·25	0·61
2·31	1·82	1·10	1·90	0·12	0·18	0·02
2·33	1·55	1·13	1·79	0·17	0·23	0·18
2·97	1·97	1·16	2·53	0·20	0·25	0·51

Mean increase in 'glucose' (col. 2 minus col. 1): 0·44.

Mean increase in unfermentable fraction: 0·065.

Mean value of glycoside glucose: 0·38 or 28 per cent. of glucose from 1.

The sugar determinations carried out may be summarized as follows:

1. Free reducing power determined in charcoal-cleared solutions for leaves and sheaths and in uncleared solutions for stems and ears, using Harding and Downs's copper reagent.

2. Free fructose as above, after oxidation of glucose by alkaline iodine.

3. Free glucose, calculated from simultaneous equations derived from 1 and 2.

4. Uncleared solutions hydrolysed with N/5 H<sub>2</sub>SO<sub>4</sub> for 10 minutes in water bath. Total glucose and fructose determined as in 1, 2, and 3, but without clarification of leaf and sheath extracts.

Increases in glucose and fructose give glucose and fructose combined as sucrose and fructosan. Estimates of sucrose can be made as twice the glucose increase, and of fructosan as the excess of fructose increase over glucose increase. These estimates may be subject to rather large errors (Archbold, 1938).

5. Uncleared solutions hydrolysed with N H<sub>2</sub>SO<sub>4</sub> for 4 hours on a water-bath, and remaining fructose and total glucose again calculated as in 1, 2, and 3.

Difference between glucose in 4 and 5 gives the apparent glycoside glucose.

6. Allowance may be made for unfermentable reducing power by correct-

ing the glucose values on the basis of Archbold's (1938) results and the results for glycosidic glucose quoted above.

#### GENERAL METABOLISM OF THE ROOT DURING THE GROWTH CYCLE

An attempt has been made to define the major changes in the root constituents during the growth cycle, and especially to ascertain whether there is any upward transference of material from the roots during ear development. Root analyses were carried out in 1938 only.

The difficulties of removing roots from soil are well recognized, and although every care was taken to minimize the loss of small roots, it became evident that increasing brittleness towards the end of the growth cycle resulted in a somewhat excessive loss at this stage. The results of the analyses of roots are presented in Table V. The fresh-weight data are to be regarded as only approximate figures owing to the difficulty of removing water after washing. During the vegetative phase root growth is active and roots contribute a high proportion to the total dry weight of the plant. While stem elongation and ear development are proceeding, root growth is virtually complete and so the proportion of root to total falls rapidly. In the present example the ratio of the dry weight of roots to that of the aerial parts was 0.44 at the end of the vegetative stage (emergence of the 10th leaf on the main axis) 68 days after sowing, and at harvest, 137 days from sowing, had fallen to 0.09.

The general drift of fresh weight (approx.), dry weight, and residual dry weight are shown in Fig. 2. All the curves show some rather large fluctuations due mainly to the technical difficulties mentioned above. It is, however, possible to say that the fresh weight reached a maximum value about 60 days after sowing (July 12, 1938), remained constant for some time, and after 80 to 90 days appeared to fall rather abruptly (Aug. 10, 1938). This somewhat sudden decline in fresh weight may be due in part to desiccation but is, to an unknown extent, certainly due to the loss of small roots, noted above. The dry weight and residual dry weight, as percentage of the fresh weight, both rose to maxima about 80 days from sowing and then remained nearly constant till harvest, with a possible tendency to rise. Soluble material, found by difference, rose from 2 to 3 per cent. of the fresh weight at the time of the emergence of the last leaf to 4 or 5 about a month later (100 days from sowing) and then remained constant. There was therefore a tendency for soluble material relative to insoluble to increase during ear emergence, but there was no relative depletion during the subsequent ripening of the ear. Sugar formed only 10 to 20 per cent. of the soluble fraction (see Fig. 2).

It is evident from the curves of Fig. 2 that if dry weight, &c., be expressed in terms of fresh weight per plant so that absolute losses may be assessed, there will be an apparent loss of dry weight from about day 90, since the fresh weight falls while the ratio of dry weight to fresh weight remains constant. The validity of the dry-weight loss thus depends on the trustworthiness of the



fresh-weight value. If the loss of fresh weight were due either to rapid desiccation or to translocation of material upwards, change in the dry-weight/fresh-weight ratio would result, so that it is concluded that much of the fresh-weight loss is due to the breaking off of small roots already discussed, and that there is no

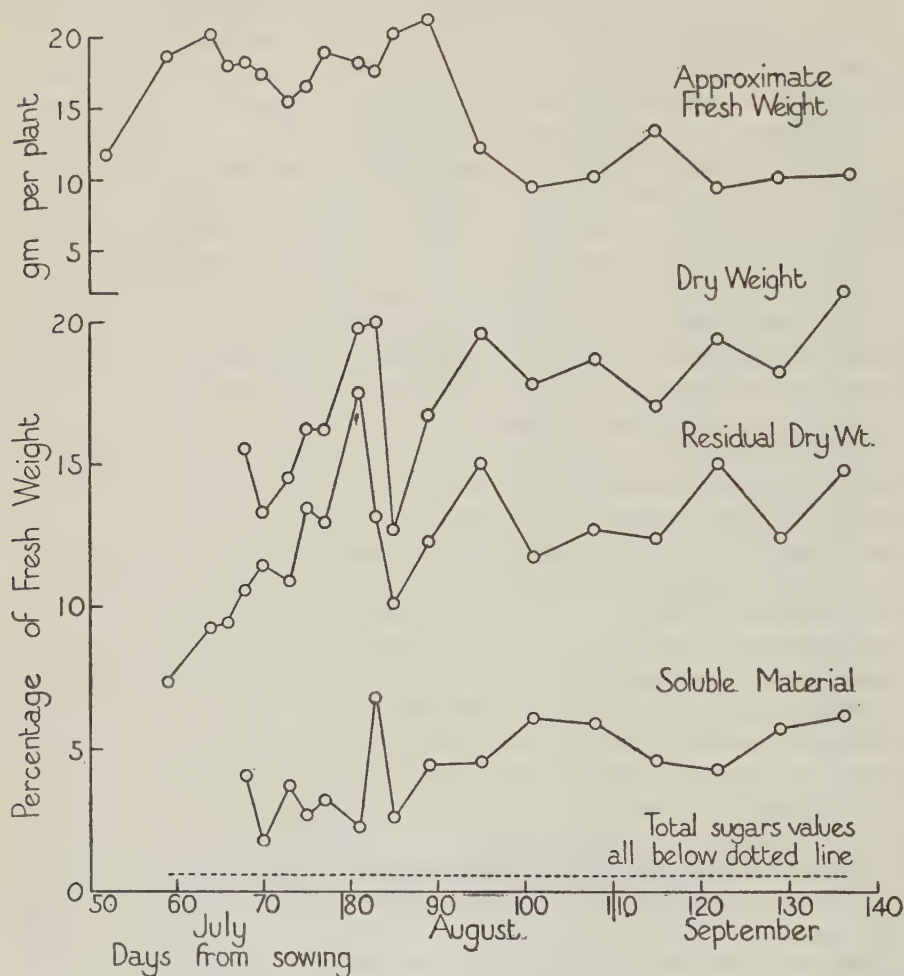


FIG. 2. Fresh weight, dry weight, residual dry weight, and total soluble material of the roots of the barley plant during growth.

appreciable transference of material to the aerial parts of the plant during ear development and ripening. In addition to the constancy of the dry-weight/fresh-weight ratio, the ratio of residual dry weight to dry weight shows no defined change after ear emergence, fluctuating round a mean of 0.73. If, therefore, it is argued that a simultaneous loss of water and transference of material upwards might result in a fall in fresh weight without change in the

TABLE V

*Composition of Barley Roots during the Second Half of the Growth Cycle 1938*

Date.	Days from sowing.	Stage of growth of main axis.	Approximate fresh weight per plant grammes.	Dry weight.	Residual dry wt.	Total sugar (after hydrolysis with N/5 acid).	Total fructose.	Total glucose.	Reducing sugar.	Unfermentable fraction (as glucose).
Percentage of fresh weight (means of duplicate determinations).										
July	4	52	11.84	—	—	0.14	0.12	0.02	—	—
"	11	59	18.60	—	7.36	0.06	0.03	0.03	0.04	—
"	16	64	20.10	—	9.19	0.14	0.11	0.03	0.01	—
"	18	66	17.89	—	9.43	0.25	0.24	0.01	0.02	0.007
"	20	68	18.25	15.58	10.56	0.20	0.20	0.00	0.02	0.007
"	22	70	17.37	13.25	11.42	0.29	0.22	0.07	0.05	0.006
"	25	73	15.47	14.50	10.79	0.24	0.22	0.02	0.01	0.009
"	27	75	16.44	16.24	13.47	0.56	0.42	0.14	0.08	0.009
"	29	77	18.74	16.13	12.93	0.55	0.40	0.15	0.09	0.017
Aug.	2	81	18.04	19.73	17.47	0.27	0.18	0.09	0.02	0.006
"	4	83	17.46	19.91	13.10	0.38	0.27	0.11	0.06	0.017
"	6	85	20.05	12.65	10.04	0.30	0.20	0.10	0.07	0.014
"	10	89	21.00	16.64	12.22	0.72	0.43	0.29	—	—
"	16	95	12.14	19.49	14.96	0.29	0.18	0.11	0.06	0.012
"	22	101	9.31	17.74	11.68	0.46	0.27	0.19	0.02	—
"	29	108	11.90	18.60	12.62	0.17	0.00	0.17	0.04	0.008
Sept.	5	115	12.99	16.94	12.32	0.21	0.00	0.21	0.07	—
"	12	122	11.03	19.26	14.92	0.07	—	—	0.02	0.006
"	19	129	11.75	18.05	12.32	0.11	0.00	0.11	0.03	—
"	27	137	9.87	20.87	14.70	0.09	—	—	0.02	0.002
Standard errors of duplicate means			1.67	1.53	0.85	0.057	—	—	0.028	—

fresh-weight/dry-weight ratio, then at the same time there would have to be a balance between possible hydrolysis of residual dry weight and loss of soluble material to maintain the constant ratio of dry to residual dry weight. In view of the behaviour of other parts of the plant where no such balance exists it seems unlikely that it should occur in the roots. If the apparent losses be accepted as real, then an estimate of the possible contribution to the ear may be made. From the time the stem starts to elongate to harvest this would amount to 10.7 per cent. of the increase in weight of the aerial parts during the same period or 7 per cent. of the harvest dry weight. This will certainly be an overestimate, since some roots are known to have been lost.

Only small amounts of sugar were found in the roots throughout the season. The highest concentration reached was 0.7 per cent. and only on three occasions did the value reach 0.5 per cent., compared with maximum values in the leaves, sheaths, stems, and ears of 3, 8, 5, and 7 per cent. respectively. A tenth to a quarter was present as reducing sugar, the range of values being 0.013 to 0.095 per cent. These values of reducing sugar were too low for satisfactory estimates of the constituent fructose and glucose to be made at the original dilutions obtained. A few samples were still further concentrated to allow of such estimations. In these the amounts of glucose and fructose were about equal, with a possible slight excess of fructose. It has already been noted that the unfermentable reducing power was equivalent to about 24 per cent. of the free reducing power when calculated as glucose. Since total fructose exceeded total glucose, the presence of fructosan is deduced.

The drift of sugar concentration with time is shown in Fig. 3. There was a general rise in concentration until ear emergence (day 81), after which reducing sugar remained constant at a low level and non-reducing sugar declined until harvest. The total amount of sugar in the root will therefore reach a maximum at ear emergence and then fall, an estimate of the magnitude of this fall (see p. 13), based on the fresh-weight values will, for the reasons put forward above for the dry weight, be too high. Most of the change in sugar was in the fructose fraction which followed closely the seasonal change of total sugar, finally falling until in September none could be detected. By the methods employed fructose and glucose cannot be separately estimated if less than 0.05 mg. of fructose is present in 5 ml., so that small amounts might still have been present at the end of the season. At the peak value the ratio of total fructose to total glucose was 3:1.

For the present purpose it is sufficient to know that the concentration of sugar in the root is always low, and that at no time is there any accumulation. Furthermore, except in the very early stages of growth (up to the fourth leaf stage) when the proportion of roots to aerial parts is greater than unity, only a small fraction of the sugar in the plant is found in the root. In the present instance the ratios of root sugar to total plant sugar were, at the third leaf stage (31 days from sowing) 0.64, at the seventh leaf stage (52 days from sowing) 0.05, at the root sugar maximum 0.06, and three weeks before harvest



0.02. During the period of ear emergence and ripening, therefore, it is established that the root sugar is a very small fraction of the whole. Between the time when root sugar is at its maximum and harvest there was a possible loss of 70 mg. per plant from the root, an amount equivalent to only 0.9 per cent.

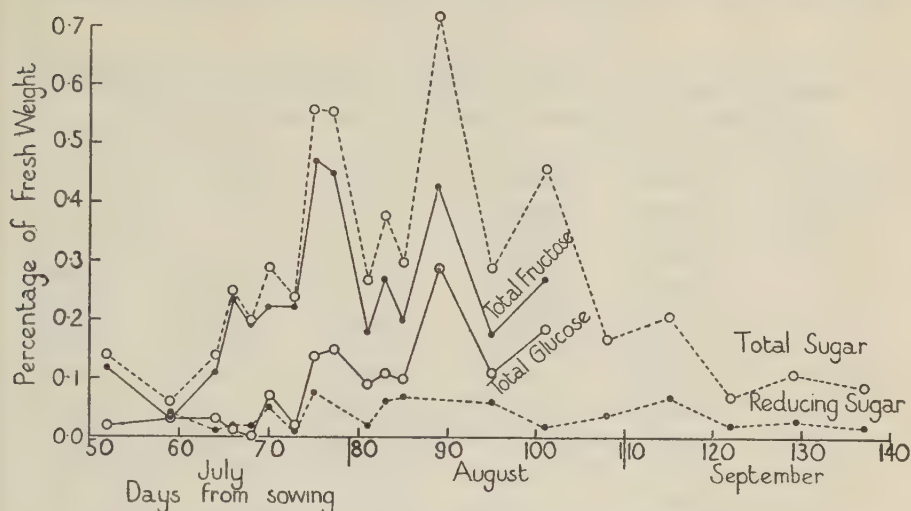


FIG. 3. Sugar concentrations in the roots of the barley plant during growth.

of the increase in weight in the aerial parts during the same period, and to 0.04 per cent. of the root dry weight per day, an amount which might easily be lost in respiration. It therefore seems certain that root sugars are quite unimportant in sugar metabolism during ear development.

On the basis of the results presented above it is assumed in the sequel that the root makes no material contribution to the carbohydrate of the ear.

#### DISTRIBUTION AND SEASONAL CHANGES IN GLYCOSIDE GLUCOSE

In a preliminary trial of the action of  $N H_2SO_4$  on barley extracts Archbold (1938) found, in a leaf extract, an increase of over 50 per cent. in the glucose produced, over that produced by hydrolysis with  $N/5$  acid, but in a stem extract no additional glucose resulted from the action of the strong acid. Before discussing the seasonal drifts of sugar in the aerial parts of the plant it is thus necessary to consider this glycoside glucose as a possible source of sugar for reutilization during growth of the ear. Barnell (1936) has followed the seasonal drift of the glycoside glucose as precipitated by basic lead acetate from alcoholic extracts of wheat shoots. In general the amounts found were rather greater than those of free glucose, and followed the same seasonal drift. The values ranged from 0.2 to 0.8 per cent. of the fresh weight, and there was a well marked fall in the amounts after the ear had 'shot'. At the maximum value the amount present was about 17 per cent. of the total of sucrose,

fructose, and glucose. Barnell regards glycoside as 'a reservoir of glucose which is drawn on by the plant in times of large sugar requirement'. In a further series of experiments Barnell (1938), dealing with the separate components of the wheat plant, found values for leaves, sheaths, stems, and ears of the order of 0.4, 0.3, 0.15, and 0.08 per cent. of the fresh weight respectively.

TABLE VI

*Glycoside Glucose (per cent. fresh weight) of Parts of Barley, 1938. Sowing Date, May 13. (Values corrected for Unfermentable Fraction)*

Days from sowing.	Leaves.	Sheaths.	Stem.	Ear.	Root.
31	0.40	0.27	—	—	—
38	0.32	0.19	—	—	—
52	0.41	0.21	—	—	0.13
68	0.44	0.11	0.05	0.09	0.08
77	0.28	0.11	0.10	0.05	0.02
89	0.27	—	0.08	0.24	0.00
101	0.43	0.21	0.24	0.68	0.00
115	0.29	0.30	0.09	0.31	0.00

mg. per plant.					
31	5	1	—	—	—
38	9	3	—	—	—
52	38	14	—	—	16
68	63	9	5	0.4	13
77	30	8	19	3	4
89	42	—	24	31	0
101	38	8	53	126	0
115	10	8	22	58	0

In the present experiment leaf samples were analysed on 21 occasions during the growth cycle, and other plant organs on 8 occasions. Some glycoside glucose was found in all parts of the plant, but the concentration in the leaves was markedly higher than elsewhere. Relative to other sugars the leaf also contained the highest amount, about 20 per cent. as compared with 5 per cent. in the stems. The values for the leaves are shown in Fig. 4 and for all parts of the plant on the 8 occasions in Table VI. There is no marked concentration drift in the leaf, the values fluctuating round a mean of 0.35 per cent. (Fig. 4). The mean values for sheaths, stems, and ears were 0.20, 0.11, and 0.27 per cent. respectively, and again there was no marked concentration drift except that there was a tendency for the glycoside glucose of the ear to rise towards harvest. In the root concentration was always low and by the end of July none could be detected. There was a possible loss of about 16 mg. during July, an amount equivalent to 0.16 per cent. of the harvest weight of the ear, so that there is clearly no glycoside reserve in the root. On a single plant basis leaf (see Fig. 4) and sheath values reached a maximum about 70 days from sowing and then fell to harvest, stem values showed no tendency to fall as the ear developed, while in the ear itself the amounts of glycoside glucose rose.

If the total amount of glycoside glucose in the plant be considered at the time of the maximum amount in the leaves, it is found to be 90 mg. or about 1 per cent. of the harvest weight of the ear. In the barley plant, therefore, there is never an accumulation of this form of sugar in any quantity which

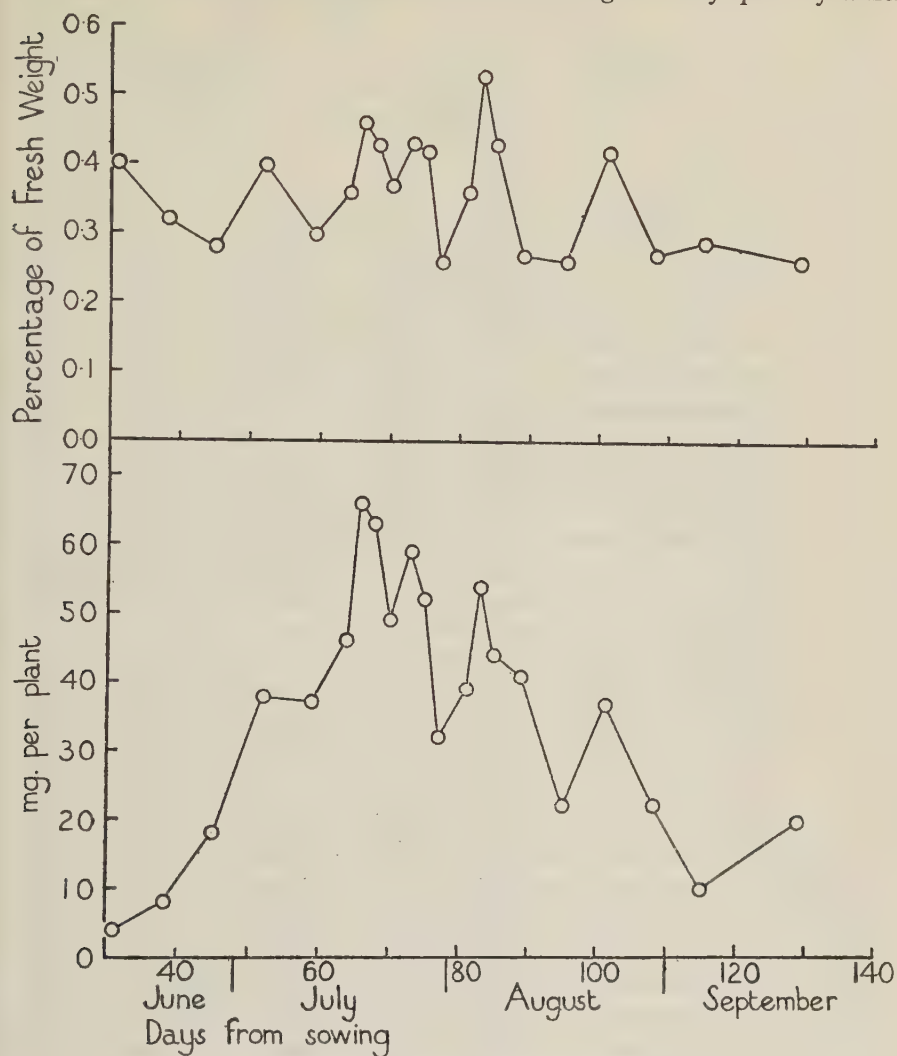


FIG. 4. Glycoside glucose in barley leaves during growth.

could form a reserve for starch synthesis in the ear, although there is evidence of a fall towards harvest. The actual loss is 60 mg., which is about 5 per cent. of the other forms of sugar lost and only 0.7 per cent. of the corresponding gain in dry weight of the aerial parts of the plant.

These results agree in general with those of Barnell for wheat, as regards the



TABLE VII

*Composition of Aerial Parts of Barley during Growth. Means of Duplicate Determinations of Samples of Three Plants Each*

Date of collection.	Days from sowing.	Stage of growth (main axis).	Water content.	Dry wt.	Residual dry wt.	Total sugar after hydrolysis	Total fructose	Total glucose
			gm. per plant.			mg. per plant.		
1938								
June 13	31	3rd leaf	1.25	0.19	—	23	14	9
" 20	38	5th "	3.47	0.53	—	67	41	26
" 27	45	6th "	8.83	1.28	—	65	41	24
July 4	52	After 7th leaf	13.61	2.62	—	259	164	95
" 11	59	" 8th "	19.93	3.89	2.33	300	170	130
" 16	64	9th leaf	22.22	4.50	3.04	592	360	232
" 18	66		24.10	5.54	3.60	770	538	232
" 20	68	10th "	26.18	6.35	4.26	1015	730	285
" 22	70		26.95	6.54	4.54	961	714	247
" 25	73		28.60	8.58	5.86	1534	1143	391
" 27	75	Awns showing	30.96	8.69	5.73	1607	1169	438
" 29	77		33.00	10.54	7.03	2144	1623	521
Aug. 2	81	Ear emergence	30.88	11.45	8.42	1566	1132	434
" 4	83		33.69	14.33	10.71	2049	1353	696
" 6	85	3 green leaves left	35.74	12.76	9.70	1824	1266	558
" 10	89		45.77	15.52	11.39	2414	1638	776
" 16	95		34.76	15.19	11.85	2019	1373	636
" 22	101	2 " "	34.27	18.01	13.15	3166	2252	914
" 29	108	All leaves dead	36.87	17.99	14.96	924	647	277
Sept. 5	115		22.37	18.76	16.06	1280	922	358
" 12	122		25.91	—	—	873	619	254
" 19	129		28.58	19.15	15.88	577	404	173
" 27	137	Harvest	18.13	—	—	—	—	—
1940								
June 28	60	Awns showing	6.84	2.17	1.32	513	470	43
July 3	65		9.11	3.22	2.09	691	608	83
" 5	67	Ear emergence	8.52	3.60	2.23	891	707	184
" 10	72		8.30	4.17	2.52	1114	880	234
" 12	74		8.16	4.54	2.81	1162	869	293
" 15	77		8.76	4.77	2.78	1485	1269	116
" 17	79		10.50	4.58	2.86	1096	920	176
" 19	81		9.80	5.14	3.24	1359	1105	254
" 22	84	3 green leaves left	10.38	5.79	3.68	1409	1160	249
" 24	86	2 " "	8.66	5.09	3.62	904	712	192
" 29	91		11.52	7.07	5.12	1149	940	209
Aug. 2	95	1 " "	10.94	7.33	5.34	1185	998	187
" 5	98		10.67	7.15	5.58	773	629	144
" 9	102	All leaves dead	10.61	7.91	—	683	565	118
" 16	109		8.33	8.24	7.08	485	390	95
" 23	116		7.11	7.49	6.53	233	198	35
Sept. 9	133		4.4	8.08	7.52	89	78	11
" 10	134		3.8	7.41	6.73	127	93	34
" 11	135		3.1	6.88	—	94	—	—
" 12	136	Harvest	2.5	6.43	5.93	67	53	14

levels of glycoside glucose in the several components and the seasonal drift in amount, except that there was no increase in the ear in wheat as in barley. From Barnell's 1938 experiment (Table V) the glycoside glucose lost from the leaves, stems, and sheaths while sugar is falling comprises about 10 per cent. of the loss of other sugars, and 1.3 per cent. of the final dry weight of the ear.

These figures are of the same order as those found here for barley, so that it would seem justifiable to conclude that glycoside glucose does not form an appreciable reserve of carbohydrate for translocation to the ear in either cereal.

#### AERIAL PARTS

Since it has been established that neither roots nor the glycoside glucose afford reserve material for starch synthesis, it remains to consider in relation to ear development changes in the main sugar fractions in the aerial parts of the plant.

Analyses of the aerial parts of the plant have been carried out during seasons 1938 and 1940. The periods covered were from emergence of the third leaf in 1938, and from ear emergence in 1940 until harvest. Water content, dry weight, residual dry weight (material insoluble in alcohol and cold water), total sugar, and its constituent fructose and glucose were observed on 20 and 21 occasions respectively. Leaves, flag leaf sheath, stems (with sheaths other than that of the flag leaf), and ears were separately analysed. The results are presented in Tables VII, VIII, and IX. In 1940 the plants were grown under conditions of partial nitrogen deficiency, in order to induce a high sugar concentration as compared with the 1938 samples. This was brought about by increasing the number of plants in a pot from 3 to 6 and reducing the application of nitrate from 6 gm. to 2 (see Table I). The seasonal difference and difference of sowing date will doubtless also make some contribution to the observed variation in composition between the two series. The maximum water contents and dry weights attained by the several organs and the ratios of leaf to stem and stem to ear for the maximum values are shown in Table X and indicate the differences in size between the two sets of plants.

The drift of water content and the progress of accumulation of dry weight and residual dry weight are shown in Figs. 5 and 6. They follow a now well established course, namely a steep rise to a maximum reached successively by leaves, sheaths, stems, and ears, followed by a decline, rapid in the case of water content and slow in the case of dry weight. The ear dry weights in 1938, however, were still rising at the last sample (137 days from sowing). The maximum water content of the shoot was reached about the 95th day and it is evident from the subsequent rapid fall in water content relative to the dry-weight change that rising concentration of plant constituents in the second half of the growth cycle is mainly due to loss of water, since although carbon assimilation continues at a decreasing rate up to about 120 days only the ear increases in dry weight while water content is falling. This period of water loss covers 40 per cent. of the whole growth cycle and it is during this time that depletion of other components by upward movement to the ear may be expected to occur. In terms of the maximum dry-weight values the losses of leaves, sheaths, and stems amount to 30, 24, and 15 to 24 per cent. respectively.

*Composition of Parts of Barley during Growth, 1938. Means*

Days from sowing.	Water content. gm. per plant.	Dry weight.	Residual dry weight.	Total sugar after hydrolysis	Total fructose with N/5 acid.	Total glucose
				mg. per plant.		
LEAVES						
31	0.83	0.14	—	19	11	8
38	2.18	0.40	—	54	32	22
45	5.46	0.92	—	48	32	16
52	7.55	1.73	0.96	158	99	59
59	10.01	2.34	1.34	140	77	63
64	10.24	2.55	1.64	294	165	129
66	11.39	3.07	1.91	297	188	109
68	11.17	3.33	2.12	303	190	113
70	10.31	2.92	1.98	216	138	78
73	10.30	3.45	2.27	377	217	160
75	9.43	3.08	2.02	323	198	125
77	8.57	2.92	1.92	298	191	107
81	7.56	3.25	2.21	215	121	94
83	6.53	3.48	2.38	288	161	127
85	7.36	2.79	2.18	180	107	73
89	11.56	3.44	2.46	227	132	95
95	6.07	2.31	1.64	169	92	77
101	5.68	2.95	1.96	408	241	167
108	6.74	2.06	1.46	43	19	24
115	0.93	2.28	1.93	42	19	23
122	—	—	—	—	—	—
129	5.36	2.22	1.66	14	0	14
Standard error of duplicate means	0.82	0.18	0.20	30	19	14
STEMS						
66	5.56	1.17	0.78	223	164	59
68	8.29	1.66	1.18	359	263	96
70	9.98	2.32	1.63	411	316	95
73	13.75	3.46	2.51	638	475	163
75	13.22	3.82	2.56	650	488	162
77	15.57	4.85	3.32	1023	779	244
81	15.08	5.11	3.89	781	588	193
83	17.67	6.55	5.01	1074	746	328
85	18.75	6.20	4.72	989	717	272
89	11.95	7.36	5.36	1315	931	384
95	16.41	6.27	4.85	1082	785	297
101	14.74	6.98	4.92	1585	1223	362
108	14.77	5.93	4.62	521	413	108
115	11.27	5.86	4.75	766	620	146
122	—	—	—	556	448	108
129	13.19	5.71	4.38	430	348	82
Standard error of duplicate means	0.51	0.30	0.25	170	142	41



## VIII

## of Duplicate Determinations on Samples of Three Plants Each

Water content.	Dry weight. gm. per plant.	Residual dry weight.	Total sugar after hydrolysis with N/5 acid.	Total fructose mg. per plant.	Total glucose
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## FLAG LEAF SHEATHS

—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
—	—	—	—	—	—
7·16	1·30	0·91	250	186	64
6·35	1·36	0·91	335	263	72
5·71	1·29	0·83	289	225	64
4·53	1·20	0·79	374	340	34
4·85	1·23	0·87	440	334	106
4·55	1·55	0·96	492	400	92
3·48	1·31	0·96	272	218	54
3·65	1·61	1·19	243	176	67
3·21	1·14	0·81	234	169	65
3·06	1·21	0·93	212	163	49
2·10	1·21	0·86	101	72	29
2·22	1·21	0·99	213	152	61
2·38	0·93	0·84	36	23	13
1·22	1·01	0·71	93	67	26
—	—	—	77	54	23
1·23	1·08	0·88	42	31	11

0·54	0·12	0·15	30	25	13
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## EARS

—	—	—	—	—	—
—	—	0·05	18	14	4
—	—	0·10	45	35	10
1·97	0·47	0·32	145	111	34
2·24	0·56	0·27	194	149	45
4·31	1·22	0·84	331	253	78
4·75	1·78	1·37	298	205	93
6·01	2·70	2·13	444	270	174
6·42	2·63	1·99	421	273	148
9·20	3·51	2·64	660	412	248
10·18	5·40	4·50	667	424	243
11·62	6·87	5·34	960	636	324
12·98	9·07	8·07	324	192	132
8·97	9·61	8·49	379	216	163
—	—	—	190	92	98
8·79	10·14	9·10	91	25	66

0·76	0·52	0·53	55	31	30
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*Composition of Parts of Barley during Growth, 1940. Means*

Days from sowing.	Water content. gm. per plant.	Dry weight.	Residual weight.	Total sugar after hydrolysis	Total fructose by N/5 acid.	Total glucose
LEAVES						
60	2.02	0.74	0.44	103	90	13
65	2.49	0.84	0.49	114	111	3
67	2.31	0.97	0.55	187	128	59
72	1.67	0.88	0.53	142	108	34
74	1.47	0.87	0.50	177	133	44
77	1.58	0.79	0.47	161	115	46
79	1.94	0.72	0.41	71	53	18
81	1.33	0.83	0.50	115	83	32
84	1.26	0.74	0.47	92	72	20
86	0.92	0.66	0.42	83	63	20
91	0.98	0.82	0.51	87	64	23
95	0.69	0.69	0.45	59	47	12
98	0.65	0.75	0.45	39	27	12
102	0.43	0.76	0.47	29	18	11
109	0.26	0.66	0.46	19	13	6
116	0.21	0.63	0.43	13	8	5
133	0.09	0.61	0.41	9	6	3
134	0.19	0.58	0.38	10	6	4
135	0.09	0.50	0.35	9	5	4
136	0.10	0.51	0.34	8	5	3
Standard error of duplicate means	0.07	0.15	0.03	28	18	13
STEMS						
60	3.51	1.05	0.65	300	274	26
65	4.46	1.58	1.07	365	342	23
67	4.43	1.80	1.15	481	422	59
72	3.96	2.03	1.19	636	520	116
74	3.90	2.19	1.40	583	440	143
77	4.26	2.47	1.44	865	820	45
79	4.69	2.11	1.28	653	573	80
81	4.54	2.37	1.47	811	686	125
84	5.05	2.84	1.68	934	795	139
86	3.93	2.27	1.49	540	443	97
91	5.18	2.89	2.00	684	589	95
95	4.68	3.05	1.86	778	698	80
98	4.51	2.43	1.76	466	408	58
102	4.69	2.52	1.79	438	391	47
109	3.91	2.45	1.75	308	268	40
116	3.90	2.03	1.66	144	128	16
133	2.59	1.96	1.66	51	44	7
134	2.18	1.77	1.54	50	44	6
135	1.96	1.77	1.43	65	48	7
136	1.48	1.50	1.36	44	37	7
Standard error of duplicate means	0.38	0.20	0.14	50	36	16

## IX

## of Duplicate Determinations on Samples of Five Plants Each

Water content.	Dry weight. gm. per plant.	Residual weight.	Total sugar after hydrolysis by N/5 acid.	Total fructose mg. per plant.	Total glucose
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## FLAG LEAF SHEATHS

0.58	0.19	0.17	68	67	1
0.80	0.31	0.19	93	74	19
0.58	0.27	0.16	87	64	23
0.87	0.44	0.26	135	111	24
0.77	0.43	0.25	149	120	29
0.76	0.40	0.22	145	117	28
0.96	0.42	0.25	127	110	17
0.88	0.44	0.25	140	120	20
0.80	0.41	0.25	117	102	15
0.64	0.35	0.24	102	87	15
1.09	0.54	0.34	137	111	26
0.91	0.47	0.31	109	94	15
0.92	0.43	0.29	77	67	10
0.90	0.41	0.32	63	54	9
0.63	0.39	0.30	40	31	9
0.49	0.36	0.30	17	16	1
0.28	0.33	0.31	8	6	2
0.24	0.33	0.29	7	5	2
0.20	0.32	0.27	5	—	—
0.17	0.26	0.23	5	3	2

0.07	0.04	0.03	10	7	3
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## EARS

0.73	0.18	0.12	47	39	8
1.36	0.50	0.36	118	81	37
1.20	0.55	0.37	135	93	42
1.80	0.81	0.54	200	140	60
2.02	1.04	0.66	251	176	75
2.16	1.11	0.66	314	216	98
2.91	1.33	0.92	245	184	61
3.05	1.51	1.02	294	216	78
3.27	1.80	1.29	266	191	75
3.07	1.82	1.47	178	119	59
4.27	2.83	2.28	241	176	65
4.66	3.12	2.72	239	159	80
4.59	3.54	3.08	190	126	64
4.59	4.21	3.59	153	100	53
3.53	4.73	4.57	118	77	41
2.51	4.47	4.14	59	45	14
1.44	5.18	5.14	20	20	0
1.14	4.72	4.52	60	38	22
0.80	4.29	4.16	14	9	5
0.74	4.15	4.00	10	7	3

0.26	0.24	0.26	7	5	3
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TABLE X

*Maximum Water Content (gm.) and Dry Weight (gm.) per Plant of the Organs of Barley in two Seasons, with Differential Nitrogen Manuring, and the Leaf/Stem and Stem/Ear Ratios of the Maximum Values.*

	1938 (full nitrogen).		1940 (partial nitrogen deficiency).	
	Max. water content and days from sowing.	Max. dry wt. and days from sowing.	Max. water content and days from sowing.	Max. dry wt. and days from sowing.
Leaf . . .	11.2 (67)	3.20 (70)	2.3 (64)	0.9 (70)
Sheath . .	—	1.30 (75)	0.9 (85)	0.4 (75)
Stem . . .	18.0 (85)	6.75 (88)	4.7 (90)	2.7 (90)
Ear . . . .	11.6 (103)	10.15 (about 130)	4.6 (99)	4.8 (120)
Aerial Parts	35.0 (95)	19.6 ( " )	10.6 (95)	7.96 (115)
Ear/total at harvest 53%, 63%.				
Leaf/stem 47%, 66%; Stem/ear 33%, 56%.				

In the case of the leaves this is certainly a maximum estimate, since with plants grown in soil it is not always possible to ensure that no part is lost of the first three or four leaves which die off early.

Residual dry weight forms a large proportion of the total and the curves are therefore very similar to those of dry weight, and it is clear from the diagrams that the bulk of this fraction in the leaves, sheaths, and stems is irreversibly synthesized and only a relatively small part can be broken down and retranslocated. The values relative to dry weight range from 56 to 75 per cent. in leaves, 60 to 85 per cent. in stems, and up to 96 per cent. in ears, in which organ the starch will be included. This ratio rises with time in all parts of the plant even when total dry weight is falling, indicating a proportionally greater loss in the soluble fraction than in the insoluble. In the ears where residual dry weight increased up to harvest, there was no loss of soluble material and the rising ratio indicates the rapid formation of starch relative to the increase in the soluble fraction.

From consideration of Table X and Figs. 5 and 6 it will be seen that the time sequence in which the successive maxima of water content and dry weight are reached is little affected by the different conditions of growth in the two seasons except in the case of the ear, although the nitrogen-deficient plants were less than half the size of the full-nitrogen series. The two series differ, apart from size, firstly in the fact that the ear 'shot' earlier in the deficient plants. Thus at the stage of maximum stem dry weight (90 days from sowing) 54 per cent. of the ear growth had been made in these plants, while in the full-nitrogen series at the same stage only 35 per cent. of the ear growth was completed. It must be remembered that this represents a greater absolute weight in the larger plants, namely 3.6 gm. per plant as compared with 2.5 gm. Secondly, the deficient plants ripened off more quickly, as is shown by the failure of the ear dry weight to show a defined maximum after

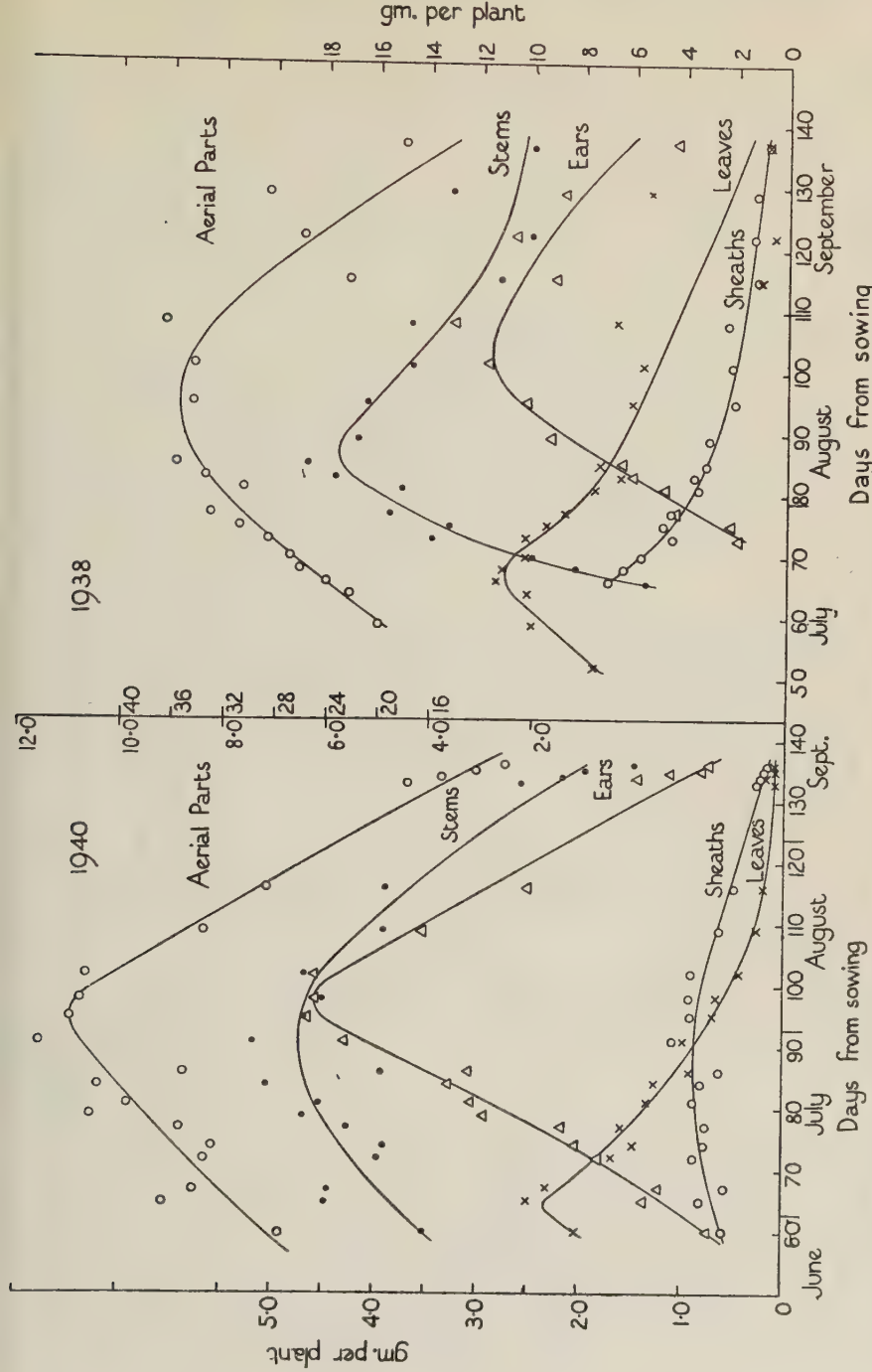


FIG. 5. Changes in water content of the organs of the barley plant during growth. The ordinate scale for the 1940 data (partial N deficiency) is four times as great as that for the 1938 data (full N), and in each year the scale for the aerial parts is half that for its component organs. The smooth curves are drawn as nearly as possible through the running means of observations on three successive dates.

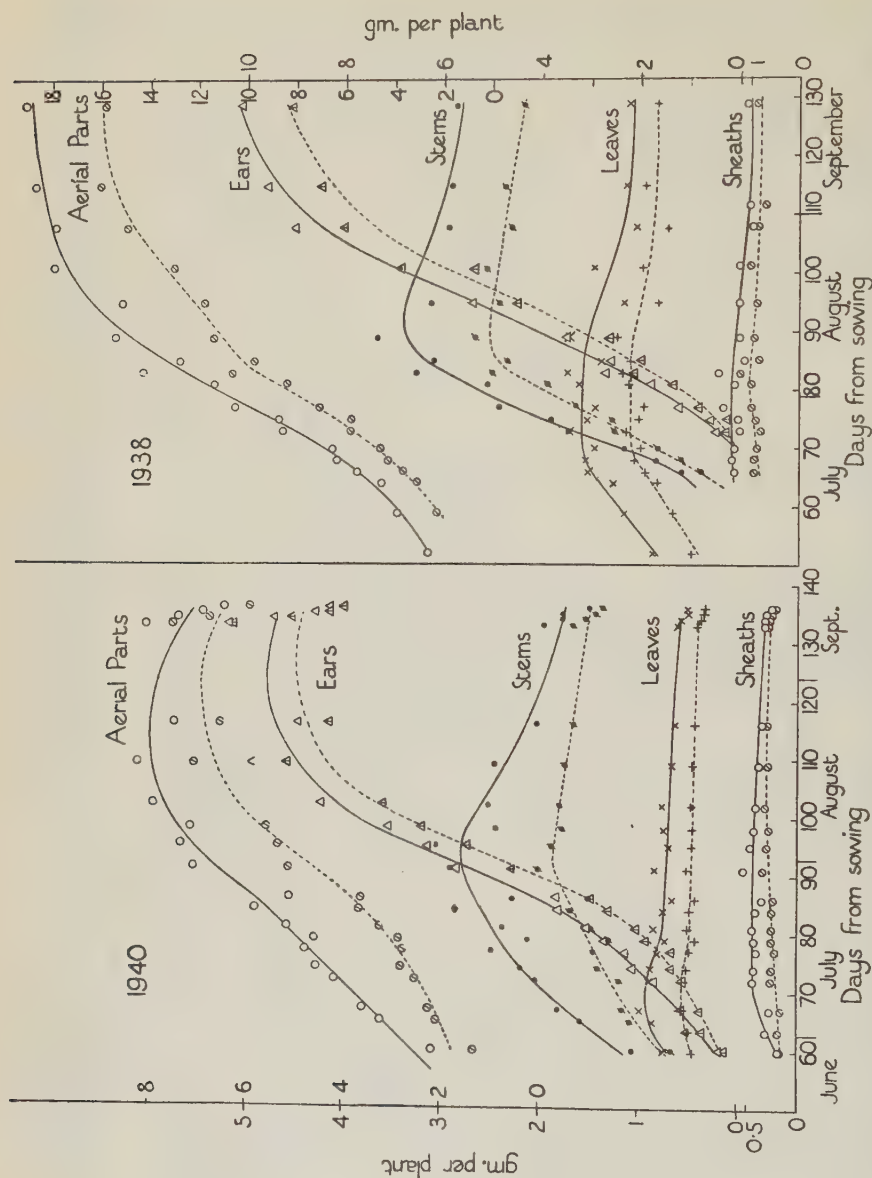


Fig. 6. Changes in dry weight and residual dry weight of the organs of the barley plant during growth. Continuous line: dry weight; broken line: residual dry weight. The ordinate scale for the 1940 data (partial N deficiency) is twice as great as that for the 1938 data (full N) and in each year the scale for the aerial parts is half that for its component organs. The smooth curves are drawn as nearly as possible through the running means of observations on three successive dates.



137 days, in 1938, while a maximum was reached in 120 days in 1940. The water contents of the ears at harvest were 46.1 (1938) and 15 per cent. (1940), showing clearly the relative immaturity of the ears at the final collection in 1938. This difference is probably in part attributable to the larger number of immature tillers on the full nitrogen plants, which remained somewhat green until the end and whose increase in weight offset the losses in the more mature tillers. Finally, the dry weight of leaf and stem was reduced by nitrogen deficiency relative to that of the ear, so that in 1940 at harvest the ear formed a higher proportion of the total dry weight.

Progress curves for total sugar, similar to those for dry weights, are shown in Fig. 7. They show the same general form, rising to maxima and then falling to harvest, with successive maxima in leaves, sheaths, stems, and ears. The curves for 1938 are flat-topped compared with those for 1940, again probably due to the higher proportion of small tillers which approach maturity in succession. In both series the stems showed the characteristic accumulation of sugar, containing at the time of the maximum 50 and 60 per cent. of the total in 1938 and 1940. The flag leaf sheath contained as much sugar as all the leaves at their maximum, so that the concentration in the sheath was high compared with that of the leaves.

In the nitrogen-deficient series of 1940 the plants showed the high concentration of sugar associated with this type of deficiency; furthermore, the time sequence at which the maxima in the several organs was reached was considerably modified as compared with the full-nitrogen series. The maximum concentrations reached are shown in Table XI. The times of these maxima do not always coincide with those of the maxima per plant.

TABLE XI

*Maximum Concentration of Sugar reached in the Organs of Barley during Growth*

		1938 (full N)		1940 (partial N deficiency)	
		Days from sowing.	% total sugar.	Days from sowing.	% total sugar.
Leaves	. .	83	2.5	74	5.5
Sheaths	. .	77	7.5	76	12.5
Stems	. .	75	6.5	76	12.5
Ears	. . .	75	6.5	76	8.0

The maximum sugar per plant occurred at day 90 in 1938, a few days before the maximum water content, but in 1940 it was reached at day 79. The difference is due to the earlier time of the maxima for stems and ears, the leaves and sheaths being unaffected. In the nitrogen-deficient plants, therefore, higher relative sugar values are reached and at an earlier date than in full-nitrogen plants. This early fall in sugar content may perhaps be related to the early 'shooting' of the ears already noted, and it is found that at the stem sugar maxima the ears in the two series had made about the same proportion of

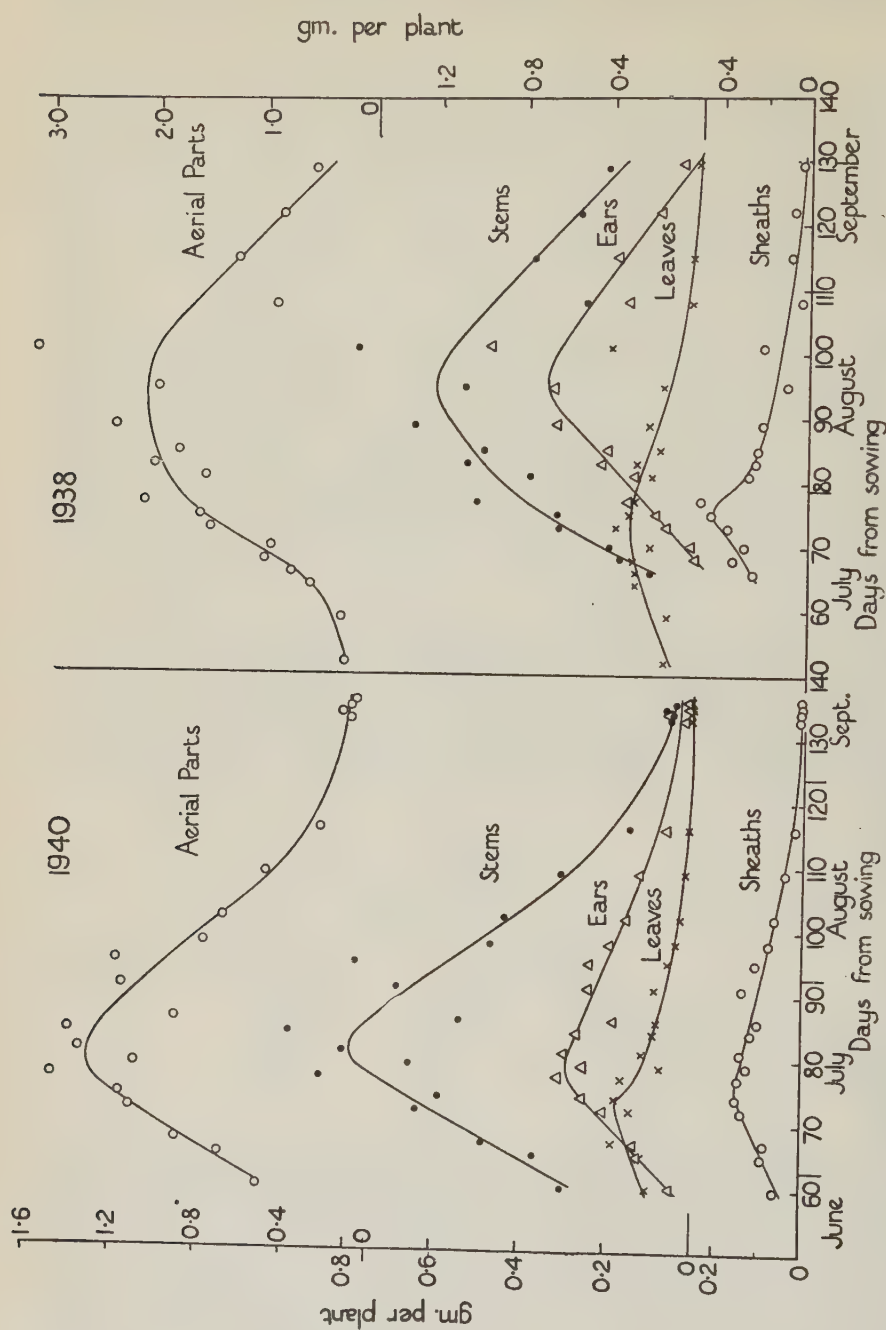


FIG. 7. Changes in total sugar content of the organs of the barley plant during growth. The ordinate scale for the 1940 data (partial N deficiency) is twice as great as that for the 1938 data (full N), and in each year the scale for the aerial parts is half that for its component organs. The smooth curves are drawn as nearly as possible through the running means of observations on three successive dates.

their total growth, namely 39 and 33 per cent. Approximately 60 per cent. of the harvest weight of the ear is thus accumulated during the period when stem sugar is falling. In the nitrogen-deficient series the stems continued to increase in weight for about 10 days after the sugar maximum had been reached, but in normal plants the stem dry weight was past its maximum before stem sugar started to fall.

In order to consider, in a quantitative manner, the changes in sugar relative to the growth of the ear smooth curves have been drawn through the plotted observations, as shown in the figures. These were obtained by using the running means of observations on three successive dates, while the points shown are means of duplicate observations on the same day, from which the running means were derived. The standard errors of these duplicate means are given in Tables VIII and IX. Fluctuation round the smooth curve is largely due to the variability in size of plant, but some part is attributable to weather conditions prior to sampling. Gregory and Baptiste (1937) found that abnormal sugar values in barley leaves were related to high or low values of sunshine before sampling and the same is true in both the present series of analyses. From the smoothed curves values may be read off giving estimates of sugar, dry weight, &c., at any desired time. For the present purpose the growth cycle may be divided into four stages:

1. From sowing to maximum leaf dry weight, a vegetative stage of 70 days.
2. From leaf dry-weight maximum to stem dry-weight maximum (stem elongation and 'shooting' of the ear), 20 days.
3. From stem dry-weight maximum until the leaves on the main axis are dead (ear alone increasing in dry weight), 12 to 18 days.
4. From end of stage 3 to harvest (ear losing water rapidly and growth rate falling to zero), about 30 days.

The values at each of these stages as determined from the smoothed curves are given in Table XII.

During the first stage all parts of the plant gain in dry weight and in total sugar, so that no translocation of stable sugars is demonstrable. At the second stage leaves and flag leaf sheath begin to lose sugar, while stems and ears increase rapidly in dry weight and in sugar. Sugar continues to increase in the stems and ears throughout this stage in the full-nitrogen plants, but in the nitrogen deficient the sugar maximum is reached before the stem dry-weight maximum. In the third stage sugar falls in all plant components and only the ear increases in dry weight, while in the fourth sugar continues to fall as the rate of dry-weight increase of the ear falls to zero. The gains of dry weight and corresponding losses of sugar, &c. are given in Table XIII.

At first loss of stored sugar represents only a small fraction of the corresponding gain in dry weight, but the proportion rises continuously, reaching 35 and 53 per cent. at stage 4 in the two series. However, at this stage the assimilation rate has fallen to a very low value and less than 20 per cent. of the



TABLE XII

*Values of Dry Weight, Residual Dry Weight, and Total Sugar in the several Organs of the Barley Plant at different Stages of Growth read off from the Smoothed Curves of Figs. 6 and 7.*

Stage.	Days from sowing.	1938.					1940.				
		Gm. per plant.									
		Leaves.	Sheaths.	Stem.	Ears.	Aerial parts.	Days from sowing.	Leaves.	Sheaths.	Stem.	Aerial parts.
1. Max. leaf dry weight .	70	3.20	1.30	2.25	0.20	6.95	70	0.90	0.38	1.94	3.90
2. Max. stem dry weight .	90	3.00	1.25	6.80	3.90	15.00	90	0.73	0.42	2.70	6.38
3. Leaves on main axis dead .	108	2.40	1.05	6.20	8.25	18.00	103	0.71	0.42	2.46	7.78
4. Max. ear dry weight .	129 about	2.20	1.00	5.65	10.15	19.10	120	0.63	0.36	2.10	7.96
4. S.E. of difference between running means of stages 2 and 4 . . . . .		0.10	0.07	0.17	0.29			0.09	0.03	0.12	0.14
Residual dry weight.											
1. Max. leaf dry weight .	70	2.10	0.85	1.75	0.10	4.75	70	0.51	0.22	1.20	2.40
2. Max. stem dry weight .	90	2.10	0.90	5.05	3.1	11.00	90	0.47	0.30	1.78	3.34
3. Leaves on main axis dead .	108	1.80	0.80	4.75	7.30	14.50	103	0.46	0.30	1.78	3.74
4. Max. ear dry weight .	129	1.70	0.80	4.35	9.00	15.90	120	0.43	0.30	1.64	6.92
4. S.E. of difference between running means of stages 2 and 4 . . . . .		0.10	0.09	0.14	0.29			0.02	0.02	0.09	0.14
Total sugar.											
1. Max. leaf dry weight .	70	0.29	0.33	0.45	0.065	1.10	70*	0.16	0.12	0.54	1.00
2. Max. stem dry weight .	90	0.19	0.17	1.19	0.62	2.14	90*	0.07	0.11	0.68	1.10
3. Leaves on main axis dead .	108	0.12	0.10	0.95	0.51	1.68	103	0.03	0.06	0.44	0.65
4. Max. ear dry weight .	129	0.03	0.04	0.42	0.09	0.57	120	0.01	0.02	0.16	0.22
4. S.E. of difference between running means of stages 2 and 4 . . . . .		0.02	0.02	0.10	0.03			0.01	0.005	0.03	0.003

\* The sugar maximum in 1940 occurs between these two dates. The maximum value for the aerial parts was 1.28 gm. per plant.

final dry weight of the ear remains to be accumulated, so that although the proportion of sugar is high the absolute amount remains small. The maximum assimilation rate occurs in stage 2 at the time of rapid stem elongation following the attainment of maximum leaf dry weight, so that the period of

TABLE XIII

*Losses of Sugar in Leaves, Stems, and Sheaths and corresponding Gains in Dry Weight of Ear at Different Stages of Growth in the Barley Plant, together with Assimilation Rates (calculated from Dry-weight Data)*

	Duration days.	Assim. rate of aerial parts (mg. per gm. dry wt. per day).	Loss of sugar.	Gain of dry wt.	Sugar loss as % of gain of ear dry wt.	% final dry wt. of ear formed during each stage.
gm. per plant. 1938 (full nitrogen).						
			Leaves and sheaths.	Stems and ears.		
Stage 1	70	27	0.00	—	—	4
„ 2	20	38	0.26	8.25	3	36
			Leaves, sheaths, and stems.	Ears.		
„ 3	18	10	0.38	4.35	9	42
„ 4	21	3	0.68	1.90	35	18
1940 (partial nitrogen deficiency).						
			Leaves and sheaths.	Stems and ears.		
„ 1	70	20	0.00	—	—	16
„ 2	20	24	0.10	2.53	4	37
			Leaves, sheaths, and stems.	Ears.		
„ 3	13	15	0.33	1.66	20	34
„ 4	17	1.3	0.34	0.64	53	13

rapid loss of sugar is associated with falling assimilation rate. It is clear from Figs. 6 and 7, however, that the rate of loss of sugar is not increased as the assimilation rate falls but remains constant in 1938 and falls in 1940, so that sugar is not drawn on at an increasing rate as assimilation fails. The relatively greater losses of sugar in the nitrogen-deficient plants are manifest from the figures and again from Table XIII. These differential losses are further discussed in the sequel.

In terms of the final dry weight of the ear, the sugar losses amount to 1.06

and 0.67 gm. per plant respectively, compared with maximum ear dry weights of 10.2 and 4.8 gm., or 10 and 14 per cent. respectively. On the assumption that all the mobile sugar is translocated to the ear the above figures represent the maximum contribution to the ear which can be made by stored sugar.

From the data of Table XII it may be concluded that there is no evidence of loss from the sheaths of material other than sugar. In 1938 there was possibly a small loss of residual dry weight, but since the sugar and total dry-weight losses are equal, this change, if real, indicates a conversion of residual dry weight to non-reducing soluble substances, and not translocation away. In the leaves and stems dry-weight loss consistently exceeds that of sugar. In the two seasons the percentage of the total loss accounted for as sugar is 26 and 90 in the leaves and 50 and 90 in the stems. In every case except that of the leaves in 1938 this additional loss was all in the residual dry-weight fraction, in fact the sum of sugar and residual dry-weight losses tends to be greater than that of dry weight. This suggests a somewhat greater rate of hydrolysis of residual dry-weight material than the rate of translocation of the material to the ear. The residual dry weight here includes alcohol denatured protein, and since it is known that in the barley plant most of the nitrogen is taken up in the first six weeks of growth and subsequently reutilized in the ear, it is suggested that these losses of residual dry weight are mainly losses of nitrogenous substances. Brenchley (1912) found that the mature ear of barley contained 1.7 per cent. of nitrogen in terms of the dry weight. On this basis the protein contents of the ears at harvest in the present instances have been calculated as 1.02 gm. in 1938 and 0.50 gm. per plant in 1940, while the losses of residual dry weight from the leaves and stems are 1.10 and 0.20 gm. per plant. It is thus possible to account for residual dry-weight losses in the manner suggested, and hence to conclude that there is no material breakdown of complex carbohydrates in the leaves and stems to provide starch in the ear. Possible translocation of breakdown products of the residual dry weight amount to 10 and 4 per cent. of the ear dry weight respectively. Adding these values to those of sugar losses the total amount which could be supplied to the ear is of the order of 20 per cent. of its final dry weight in both series. At least 80 per cent. of the material in the ear must therefore result from direct assimilation, and the contention of D  h  rain and Dupont that such assimilation by organs other than the leaves, and late in the growth cycle, is an important factor in starch storage receives indirect confirmation.

The sugar losses in the ears themselves amount to only 5 and 3 per cent. of the final dry weights. Considered in relation to the gains of dry weight and residual dry weight, it is by no means certain that even this sugar is really condensed to starch. The relevant data are presented in Table XIV. There is evidently no significant difference in either series between the gains of dry weight and residual dry weight, the value for dry weight being a little higher in one and a little lower in the other. If the sugar is converted to starch and is thus included in the residual dry weight, then there must be a



compensating gain of non-reducing soluble material. If, however, the sugar were used in respiration the result would be a rise in the ratio of residual dry weight to dry weight, which it has already been stated is in fact the case (see p. 22), but this, too, would require a compensating increase of non-reducing

TABLE XIV

*Changes in Dry Weight, Residual Dry Weight, and Total Sugar (gm. per Plant) in the Ears of Barley during Growth*

	1938.	1940.
Loss of sugar . . . . .	0.53 ± 0.04	0.15 ± 0.004
Gain of dry weight . . . . .	6.25 ± 0.48	2.30 ± 0.14
„ residual dry weight . . . . .	5.90 ± 0.46	2.50 ± 0.15

soluble substance, since the total soluble fraction does not fall. The third possibility is the conversion of sugar directly to non-reducing soluble substances. Although the fate of this sugar is uncertain, it can safely be concluded that it is quite unimportant as a possible precursor of starch.

#### CHANGES IN FRUCTOSE AND GLUCOSE

The seasonal changes in total fructose and glucose are shown in Fig. 8. Data for free fructose and glucose were collected, but as these always formed but a small proportion of the whole and the amounts changed very little they do not call for detailed discussion. The observed changes may be taken to be mainly changes in fructose and glucose combined in sucrose and in fructosans. Fructose was always present in excess of glucose, indicating that some fructosans were present throughout the plant. The ratio of fructose to glucose varies in the different parts of the plant and is higher in nitrogen-deficient plants than in corresponding parts of full-nitrogen plants. High ratios of fructose to glucose are thus associated with the high sugar concentrations in the deficient plants, and this is also true of the separate organs of the plants of each treatment. Stems and sheaths, with high sugar concentrations, thus have a higher ratio of fructose to glucose than leaves and ears in which sugar concentration is lower. In leaves, sheaths, and ears the ratio tends to fall with time, but in the stems it tends to rise. The values of the ratios are given in Table XV.

TABLE XV

*Fructose/Glucose Ratios in the Barley Plant*

	1938 (full nitrogen).		1940 (partial nitrogen deficiency).	
	At the sugar maximum of each organ.	At harvest.	At the sugar maximum of each organ.	At harvest.
Leaves . . . . .	1.5	1.0	3.0	1.5
Sheaths . . . . .	4.8	2.0	6.6	3.6
Stems . . . . .	3.0	3.8	5.0	8.0
Ears . . . . .	2.3	1.8	2.3	1.8

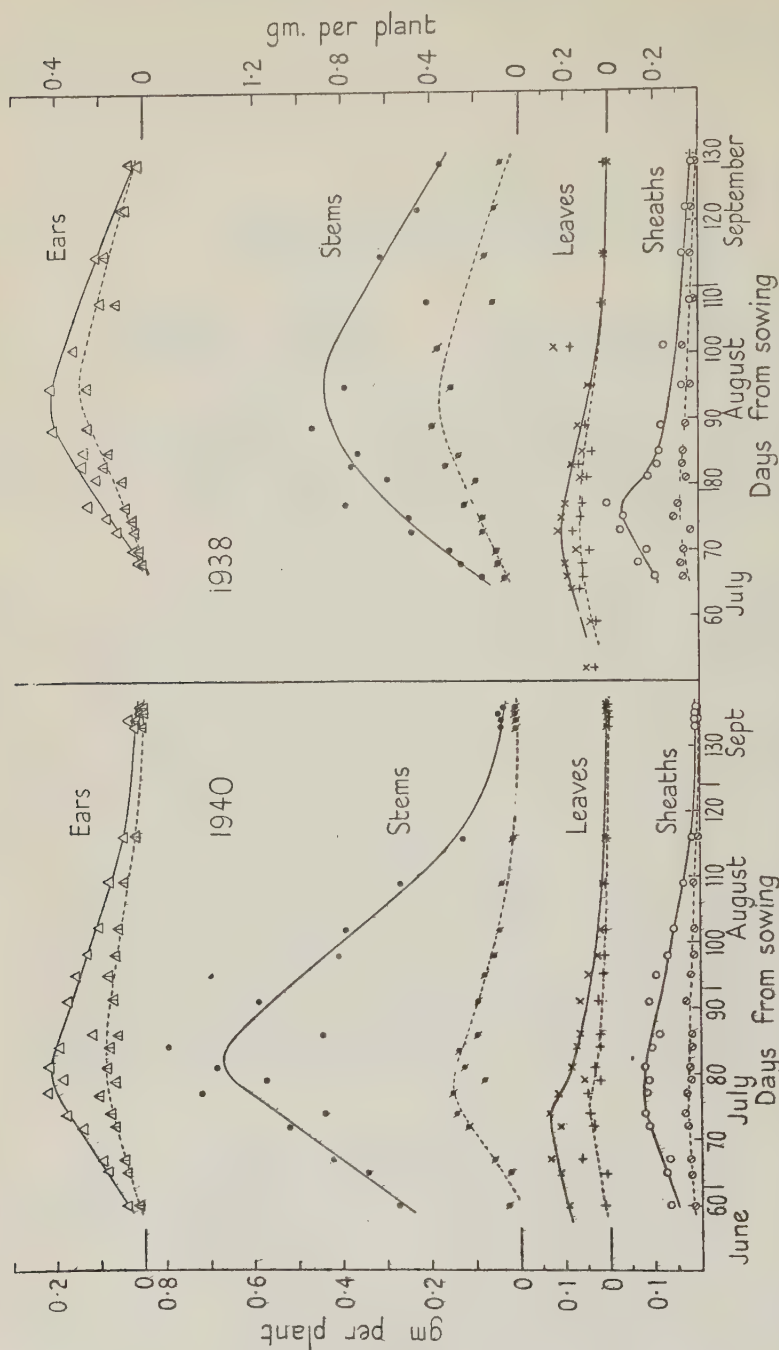


FIG. 8. Changes in total fructose and total glucose in the organs of the barley plant during growth. Continuous line: fructose; broken line: glucose. The ordinate scale for the 1940 data (partial N deficiency) is twice as great as that for the 1938 data (full N), and in each year the scale for the aerial parts is half that for its component organs. The smooth curves are drawn as nearly as possible through the running means of observations on three successive dates.

From Fig. 8 and the ratios of Table XV it is evident that the absolute loss of fructose is always far in excess of that of glucose and with the exception of the stems the percentage loss of fructose is always greater than that of glucose. There is thus a wide range of fluctuation in fructose, while that of glucose is limited. Nevertheless, both sugars show the same seasonal trend in each of the plant organs, rising to a maximum as the plant part reaches its maximum dry weight and then falling. Further, the two sugars always rise and fall together and on no occasion is the rise of one associated with the fall of the other. The changes in ratio are therefore due to a relatively faster increase or decrease of one or the other and do not indicate conversion of one to another. No evidence is forthcoming from these experiments of interconversion of these sugars, but such change at the meristems prior to further metabolism is not, of course, precluded. From the large excess loss of fructose, 66 per cent. of the total in stems, it is clear that fructosans can be further metabolized, although the nature of the further change is still uncertain. If such change does involve translocation, however, it would appear likely that fructosan is not hydrolysed first. While it is not proposed to discuss here the bearing of these results on the problems of sugar accumulation, it may perhaps be noted that the view that fructosans are formed from pre-existing reducing sugars at a relatively slow rate must be abandoned, the complex equilibria between the several sugars appear, on the contrary, to be rapidly reached.

#### DISCUSSION

It is commonly believed that sugar is stored in cereal stems before the ear 'shoots' and is subsequently translocated to the developing ear, there acting as the precursor of starch. This view is based on a well established sequence of concentration changes, namely a rapid rise during stem elongation followed by a steady fall to harvest. The maximum concentrations reached vary with the conditions of growth and may be as high as 12 to 14 per cent. At first the maximum concentration is at the base of the stem, but as successive internodes reach maturity the region of maximum concentration shifts upwards and in the top internode the sugar finally reaches a higher level than that previously obtaining at the base. In addition the ear itself has a high sugar concentration just before emergence; this gradually falls to harvest and it is assumed that the sugar is condensed to starch. This series of changes has been interpreted as an upward movement of sugar to the ear, with the implication that the amount so translocated was of importance in the total growth made by the ear. Some doubt arose as to the importance of the sugar change when data became available of the associated changes in dry weight, from which it appeared likely that the loss of sugar might be quite small in relation to the dry-weight increase of the ear. From the data presented here, which were collected with the aim of expressing these changes on a quantitative basis, not dependent on concentration, it is established that the loss of sugar from the stems is equivalent to about 10 per cent. of the harvest dry weight of the ear. Two levels of

sugar were obtained by varying the nitrogen manuring of the plants, and at the higher sugar level induced by partial nitrogen deficiency the amount of sugar lost constituted a slightly higher percentage of the ear dry weight than that lost at the lower sugar level. There is an additional loss of material from the fraction insoluble in alcohol and cold water, equivalent to another 10 per cent. only, so that a major breakdown in the cellulose complexes is precluded. Since in fact this loss in residual material may be entirely due to translocation of nitrogenous substances, there need be no reutilization of the cellulose fraction at all.

The roots do not supply reserve substances for upward translocation, neither is there any substantial breakdown of soluble glycosides, releasing glucose, in any part of the plant. As far as ear development is concerned, therefore, if the substances lost from the leaves, stems, and sheaths of the plant were all translocated to the ear a maximum of 20 per cent. of the ear dry weight would result from such depletion. It follows that at least 80 per cent. of the final weight of the ear must result from direct assimilation. In spite of the high concentrations of sugar which may be reached in the barley stem, the amounts of sugar there present are small in relation to the total material accumulated in the ear, which at harvest comprises over 50 per cent. of the dry weight of the aerial parts.

The source of this assimilate, whether from leaves, stems, or the ears themselves, will be discussed in detail in a subsequent paper, now in course of preparation. It is here proposed to consider further the fate of the stored sugar; brief reference will, however, be made in the following discussion to some of the results of sugar analysis in the experiments dealing with the separate contributions of leaves, stems, &c., to total assimilation.

From the foregoing considerations it may be concluded that ear development in barley is in no way dependent on the accumulation of sugar in the stem. It has in fact already been shown (Archbold, 1938*a*) that if a plant is defoliated as the last internode is elongating and before the bulk of the sugar has been stored up, further sugar storage is prevented; but ear development proceeds quite satisfactorily although at a reduced rate. Alternatively, if the ears are removed, there is an immediate increase in the stored sugar, indicating that the greater part of the primary assimilate is normally moved immediately to the ear. The generally accepted view may therefore be held, namely, that the assimilating surfaces produce carbohydrate in excess of that capable of immediate use in further metabolism and this excess appears as sugar in the stems. Early defoliation so reduces the assimilating surface that insufficient material is available for normal ear development, and no excess sugar is therefore stored. If, however, plants are defoliated when the sugar storage is completed, or nearly so, there is no rapid depletion of stem sugar to make good the absence of the leaves, sugar loss continuing in a manner similar to that in untreated plants (Archbold, in course of publication). Ear development proceeds, except on tillers still at an early stage of development, and



ears of normal size are produced, so that at this stage the stems and ears of the more mature tillers have between them an assimilating capacity adequate to maintain ear growth. If now the contribution in assimilation of the ear itself is eliminated by shading, the ears do not reach their full size, but again there is no abnormal rate of loss of the stored sugar. Artificial modifications in the conditions of growth, such as those mentioned above, do not therefore induce rapid loss of stored sugar to replace the deficiency in assimilation. No continuous replacement by stored sugar would, of course, be possible, since in any case in the normal plant all the sugar disappears by harvest.

Diminishing the assimilatory capacity of the plant may thus prevent sugar accumulation, but there is no evidence for accelerated utilization, and it is therefore unlikely that the demands of the ear exert any special control over movement of stored sugar from the stem.

In the series of plants grown under partial nitrogen deficiency the reduction of ear and stem growth due to lack of nitrogen results in a relatively greater excess of sugar than if nitrogen is plentiful, since this deficiency has little or no effect on assimilation rate (Gregory and Sen, 1937). If stored sugar is normally translocated to the ear then, when the growth of the ear is limited by nitrogen supply and assimilation rate maintained at the normal level, a low rate of sugar consumption would be expected, since the demands of the ear will be lowered while the reserve of sugar is greater, as compared with consumption in plants not so limited. Actually, the reverse is found, sugar being lost at a greater rate from the nitrogen-deficient plants than from normal plants, which would appear to support the view that sugar loss is independent of the demands of the ear. The accumulation of sugar, on the other hand, appears quite certainly dependent on the conditions attending ear growth, for in addition to the arguments put forward above it will be remembered that the early shooting of the ear under nitrogen deficiency prevented further accumulation of sugar in the stem and resulted in the sugar maximum being reached at an earlier date than in full-nitrogen plants.

It is, of course, an assumption that sugar disappearing from the stem is necessarily translocated to the ear, and if the loss is in fact not dependent on the requirements of the ear other possibilities have to be considered. The most obvious is that the sugar is used in respiration of the stems, leaves, and sheaths themselves. On this assumption respiration losses may be calculated from the sugar losses, using the appropriate hours of darkness. The relevant periods ranged from July to September when the hours of darkness (sunset to sunrise) increase from about 8 to 12. The results of such calculations for the periods of falling sugar for each plant component are shown in Table XVI.

The respiration rate at 25° C. for barley leaves during the vegetative stage of growth, from plants grown in sand culture with and without full nitrogen supply, ranges from 3 to 9 mg. per gm. dry weight per hour according to the experiments of Gregory and Sen (1937), values far higher than those cal-

TABLE XVI

*Sugar Losses from the Component Parts of the Barley Plant calculated in Terms of the Equivalent Carbon Dioxide, assuming Sugar is lost in Respiration during the Dark Hours.*

Sugar loss (mg. of CO <sub>2</sub> per gm. dry weight per hour).					
1938 (full nitrogen).			1940 (partial nitrogen deficiency).		
	Days from sowing.			Days from sowing.	
Leaves . . .	70-108	0.21		75-120	0.83
Sheaths . . .	70-108	0.84		75-120	1.07
Stems . . .	90-129	0.19		82-120	0.40
Ears . . .	95-129	0.33		82-120	0.28

culated above. These calculated values are, therefore, not perhaps higher than might be expected as a result of respiration, even if liberal allowance is made for the high temperatures at which Gregory and Sen's observations were made as compared with night temperatures in late summer, and also for the fact that respiration rate will fall as the plant ages. Nevertheless, obvious difficulties present themselves in accepting this interpretation of the sugar loss. Firstly, it is not clear why rates of loss of sugar from the leaf sheaths should be so high compared with that of the leaves if respiration alone is the cause of sugar loss. If concentration were deemed a factor in controlling respiration rate, then the sheaths with their high sugar concentration might be expected to respire faster than the leaves, but on the other hand the stems with equally high concentrations show no such high rates of loss. There is thus no simple relation between rate of loss and concentration; moreover, to attribute a controlling influence on respiration to sugar concentration is in conflict with the observations of Gregory and Sen (*loc. cit.*) who demonstrated conclusively that rate of respiration was not related to total sugar concentration except in cases of extreme potassium deficiency. Secondly, when the two series of plants are compared, it is seen that the rates of loss in the nitrogen-deficient plants are the higher, while it is well established that nitrogen deficiency lowers respiration rate. Loss of sugar in respiration does not, therefore, offer a completely satisfactory explanation of the facts, although clearly much of the sugar might be lost in this way. It is, of course, recognized that a definite conclusion requires direct respiration measurements, more especially as no allowance is here made for differences in temperature between the two seasons, all the difference in rates of loss being attributed to the manurial difference.

If there is no definite migration to the ear and the stored sugar is not all lost by respiration, it is still possible that there is some local movement of sugar from leaf to sheath and from sheath to the corresponding stem internode. In this connexion some further evidence relating to sugar changes in the separate internodes may be cited. It has been shown (Archbold, 1940, Barnell, 1938) that a sugar maximum is reached at successive dates in each

stem internode beginning at the base, and this time sequence has been confirmed in the 1938 series of plants. Now the leaf sheaths develop before the corresponding stem internode elongates, so that accumulation of sugar in the

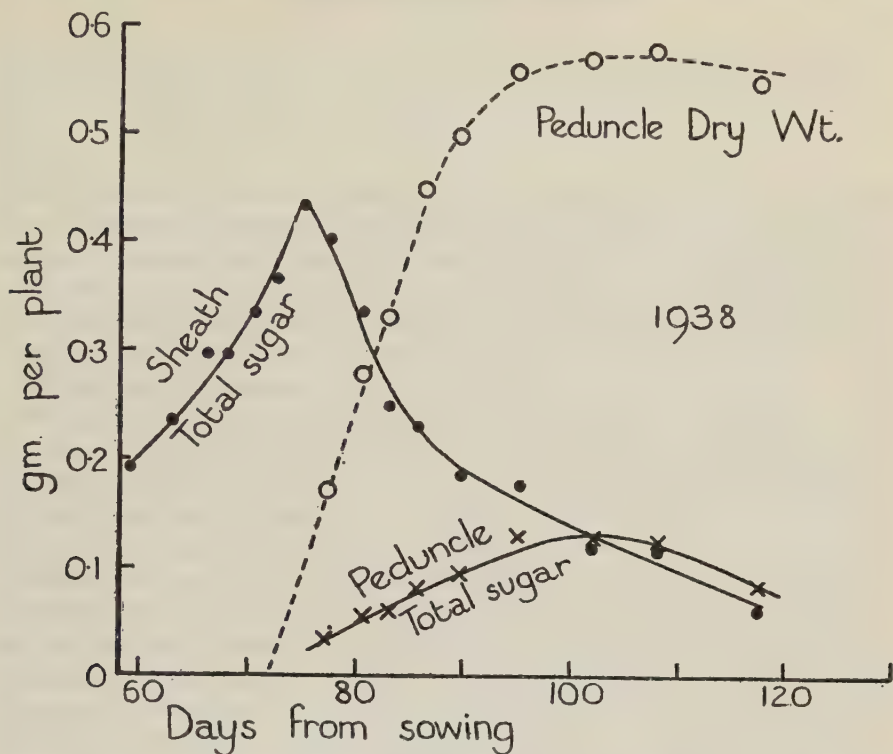


FIG. 9. Increase in dry weight and total sugar of the peduncle of the ear during growth and simultaneous fall of total sugar in the flag leaf sheath.

sheath may be the result of a local excess of sugar delivered by the accompanying leaf. When the internode grows, the sugar in the sheath is falling and there may then be a leakage of some of the excess sugar into the stem. Changes in the sugar of the flag leaf sheath and the corresponding peduncle illustrating this possibility are shown in Fig. 9. Some further support is given to this view by an analysis of the four stem internodes below the peduncle, and their attached leaf sheaths at a time a little before the top internode reached its maximum sugar content. These figures are given in Table XVII. Here a consistent fall in the sugar level of the sheaths was found from top to base, and since the internodes fall in absolute weight from top to bottom, this will correspond to an even bigger fall in absolute amount of sugar. The older (lower) sheaths are thus depleted of sugar before the younger (upper) ones, and it may be suggested, on analogy with the behaviour of the flag leaf sheath, that each is depleted as its attached internode elongates. If this is so, then the



TABLE XVII

*Total Sugar Contents (per cent. Fresh Weight) of the Internodes and Leaf Sheaths of Barley Stems. 1941*

				Leaf sheath.	Stem internode.
Internode 1 (top)	.	.	.	2.46	4.88
" 2	.	.	.	2.23	8.41
" 3	.	.	.	1.53	8.57
" 4	.	.	.	0.68	8.28

problem resolves itself into the fate of the sugar in the stem internodes only. The distribution of sugar in the stem, with the successive maxima in each internode as it reaches maturity suggests that each is dependent on the attendant leaf and sheath for a supply of assimilate and that the sugar accumulating in any one internode is that not required for local growth. It is doubtful if there is transference of this stabilized sugar from one internode to another, although the fact that sugar in the basal internodes may be falling while that in the upper ones is still rising might indicate that such transference does occur. The low level of sugar in the top internode (Table XVII), however, shows that rapid migration from the region of high sugar at the base toward the low level at the top does not occur, and it is perhaps more likely that the fall in sugar in each internode is associated with the senescence of the corresponding leaf and sheath and consequent cessation of the inflow of new assimilate. If it is not translocated upwards, the ultimate fate of the sugar, or that part of it which is not respired, would appear to be conversion to non-reducing soluble substances. The data available do not permit of detailed consideration of this possibility, but it will be recalled that the relationships of sugar and soluble material in the ear could be accounted for in this way, and indeed throughout the plant the soluble material other than sugar tends to rise with time.

Finally, it must be admitted that the observed distribution of fructose and glucose does not at present contribute to the elucidation of the problem as to the exact fate of the sugar. It does show quite conclusively, however, that there is no interconversion between the sugars and, therefore, any change in the type of sugar is localized at the site of its further metabolism and must occur immediately prior to such further change and at an equal rate. In the barley plant the storage sugars appear to be very stable, and all the evidence favours the view that they result from an excess of assimilate above that required for a growth which is controlled by a complex of other factors. Once the sugar is 'stabilized' it would appear that movement from one organ to another is slow if indeed it occurs at all, and is in any case quite unimportant for the development of the plant, which depends on the rapid use of primary assimilate. The view that sugar may be stored in one part of the plant for subsequent use in another is therefore not applicable to barley, and though no final decision can be reached as to what actually happens to the



stored sugar, it is at least certain that it is not a factor of importance in ear development.

### SUMMARY

Analyses of the organs of the barley plant have been carried out in two seasons, with the object of determining how far the developing ear is supplied by depletion of materials from the rest of the plant and in particular the part played by the sugar stored in the stem. Two levels of sugar were obtained by varying the nitrogen supply.

It is shown that there is no material interchange of carbohydrate between the roots and the aerial parts during the development of the ear, so that the roots do not constitute a reserve for the ear. The amount of soluble glycoside, which was found to be highest in the leaves, fell towards harvest, but the loss was quite insignificant in relation to ear growth, so that this possible source of supply to the ear is also excluded.

In order to estimate the major changes in the aerial parts smooth curves are drawn through observations of dry weight, residual dry weight, and total sugar for each plant organ, using running means of the data for each three successive samples. From these curves values for each constituent at selected times are read off, and from these values estimates of gains and losses during any desired period are obtained.

During the period of falling stem sugar (40 per cent. of the whole growth cycle) 60 per cent. of the ear growth was made, but the loss of sugar can account for only about 10 per cent. of the final dry weight of the ear. In addition to the sugar loss there is a loss of an amount equivalent to a further 10 per cent. from the fraction insoluble in alcohol and cold water. There is therefore no major breakdown of cellulose complexes to supply the ear, and it is suggested that this loss of insoluble material is in fact not of carbohydrate but of nitrogen compounds. At least eighty per cent. of the ear dry weight must therefore result from direct assimilation by the leaves, the stems, and the ears themselves, and stored sugar cannot be regarded as essential to ear development.

The sugar in the immature ear amounts to only 5 per cent. of its final weight, and it is doubtful if even this amount is actually condensed to starch, since there is no fall in the total amount of soluble material in the ear.

The possible fate of the stored sugar is discussed, and it is suggested that the demands of the ear exercise no special control over loss of stored sugar, especially as plants defoliated early develop ears in the absence of stored sugar, while in the presence of stored sugar the rate of sugar loss is not accelerated when the assimilating surface is diminished by defoliation or shading the ear.

The rates of sugar loss observed are probably not higher than might be expected if the sugar was lost in respiration, but the rapid loss from leaf sheaths relative to leaves and from nitrogen-deficient plants relative to full-

nitrogen plants, which facts are not in accordance with existing knowledge of respiratory losses, makes it difficult to accept this as a complete explanation of all the sugar loss.

It is suggested that each internode acts as a separate unit receiving supplies from the attendant leaf and sheath, and there may then be some movement of stabilized sugar from sheath to corresponding internode, but it is doubtful if there is transference between the internodes themselves.

There is no evidence of any conversion of fructose to glucose or vice versa. Both sugars show the same seasonal trend, rising and falling together, although the greater part of the change is in fructose.

Although the precise fate of the stored sugar remains uncertain, it is demonstrated that it plays no important part in ear development, and it is in fact not yet certain that there is any upward translocation of such sugar at all.

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# Studies in Flower Structure

## VII. On the Gynaeceum of *Reseda*, with a Consideration of Paracarpy

BY

AGNES ARBER

With two Figures in the Text

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### I. INTRODUCTION

THE question has recently been raised as to the exact route by which the pollen-tube may enter the gynaeceum of *Reseda*, which remains open at the apex from youth to maturity. Special attention has also been attracted to the carpellary analysis of the genus, in connexion with Troll's theory of paracarpy. It has hence seemed worth while to re-examine the structure of the *Reseda* gynaeceum with these relations in mind. The theoretical questions involved will be discussed after certain relevant features in the construction have been briefly described.

### 2. DESCRIPTION

A four-carpelled fruit of *Reseda odorata* L. is seen from above in Fig. 1 A. The top of the ovary is partially closed by four incurved triangular flaps, each of which is formed from the fused margins of two adjacent carpels; the arrows indicate the boundaries between the carpels. Fig. 1, B and C, show external views of the gynaeceum of *R. lutea* L., while Fig. D, a segment cut tangentially from the fruit of *R. luteola* L. and viewed from the inside, indicates the situation of the turn-over flap which occurs above each placenta. Fig. 1, E1-E5, represent transverse sections from a series through the apical region of a gynaeceum of *R. alba*, in which the transmitting tissue (dotted) can be traced from the cavity of the ovary to the stigmas. The midrib regions of the four carpels are lettered A, B, C, D, and their marginal regions, A', A'; B', B'; C', C'; D', D'. Carpels C and D are cut at a slightly higher level than A and B. The uppermost ovule borne by the placenta marked A'+B' is seen in Fig. 1, E2. At this level the tip of the corresponding downwardly directed triangular flap projects freely into the ovary cavity, and it is seen to contain a single transmitting canal (dotted). At a slightly lower level (E1) the transmitting tissue in the flap is seen to have reached the actual surface, so that it would

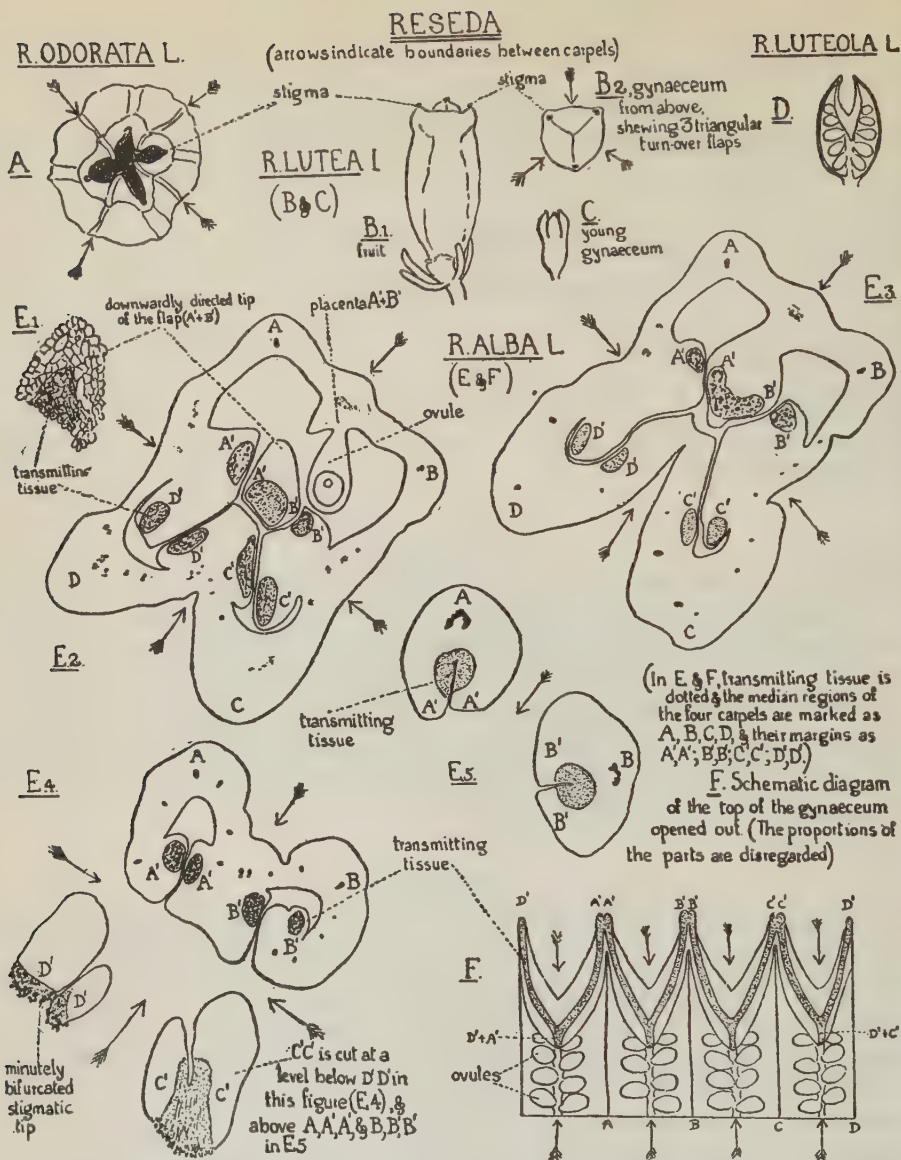


FIG. 1. A-F. Gynaeceum of *Reseda*. Arrows indicate boundaries of carpels. A, *R. odorata* L., giant-flowered variety; fruit seen from above (enlarged); open cavity of ovary shaded. B and C, *R. lutea* L. B<sub>1</sub>, side view of a fruit about 9 mm. long; B<sub>2</sub>, the same fruit seen from above. C, young gynaeceum nearly 3 mm. long. D, *R. luteola* L., segment cut tangentially from a fruit and viewed from the inside, including one placenta and the downward triangular flap on the same radius (enlarged). E and F, *R. alba* L. E<sub>2</sub>-E<sub>5</sub>, sections ( $\times 47$ ) from a transverse series upwards through the upper part of a gynaeceum. A, B, C, D, median regions of the four carpels; A', A'; B', B'; C', C'; D', D'; their marginal regions. In E<sub>2</sub>, carpels C and D are cut at a slightly higher level than A and B. E<sub>1</sub>, the tip of the downward flap formed by the adjacent margins, A' and B', of carpels A and B in E<sub>2</sub>, at a slightly lower level than E<sub>2</sub> ( $\times 77$  circa); the flap reaches to  $70\mu$  below E<sub>1</sub>. F, diagram to show the course of the transmitting canals in the triangular apical flaps of the carpels; no attempt has been made to preserve the proportions of the parts.

allow a free passage for the pollen-tubes into the interior of the ovary in the neighbourhood of the placenta. When followed up through Fig. 1, E3 and E4, the single transmitting canal is found to separate into two canals, belonging respectively to one margin of carpel A and one margin of carpel B. At a still higher level the canals of the pair belonging to each carpel approximate and eventually fuse (A'A' and B'B' in E5). When the top of the stigma is approached, the transmitting tissue makes free contact with the exterior. As the midrib region does not reach quite to the tip, at the extreme apex there is a slight bifurcation, since nothing is present but the tissue of the two margins (D'D' in Fig. 1, E4).

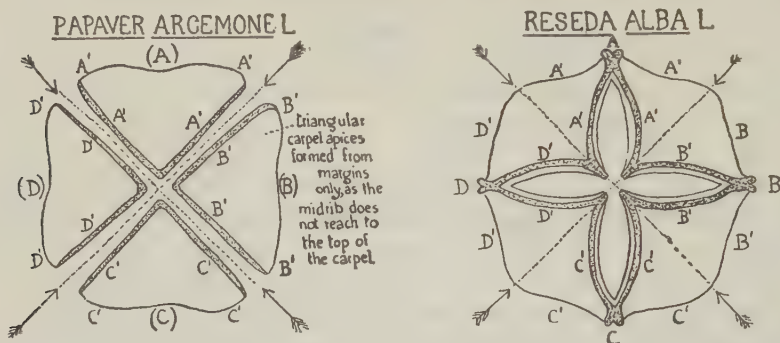
The complex structure of the gynaecium apex, which has just been described, is not altogether easy to visualize from serial sections, so the purely schematic diagram F, is included to show the course of the transmitting canals in the united marginal 'turn-overs' of the carpels. Each canal can be seen fusing at the stigmatic apex with the other marginal canal of the same carpel, while it fuses with the adjacent canal, belonging to the neighbouring carpel, towards the base of the downward flap.

### 3. DISCUSSION

In 1937 Schaeppi published a comparative study of the gynaecium in the Resedaceae, but his account of the transmitting canals is not altogether clear. Another paper belonging to the same year, by Hunt, dealing with the problems presented by styles and stigmas, also includes a consideration of *Reseda*. In describing the downward flaps, or 'fused pairs of ventral lobes', which more or less close the mouth of the gynaecium, Hunt makes his only reference to the transmitting tissue in the following terms: 'These downward lobes possess papillae which secrete the stigmatic fluid—a function which indicates that they were once upright, in a position to intercept pollen.' It is difficult to understand how this interpretation has been reached. Each transmitting canal, in fact, runs a normal, if somewhat complex course, from its freely exposed region at the actual stigma, down through the carpellary tissues, finally opening into the ovary cavity above a placenta. Though I have not observed pollen-tubes, there can, I think, be no doubt about the identification of the transmitting tissue, which is entirely typical in structure. If, as Hunt suggests, the ventral lobes once stood upright, their transmitting canals must then have been functionless, since they would not have led to the ovary cavity. As the diagrams in Fig. 1 indicate, the fact that the gynaecium in *Reseda* remains open at the top in no way affects the route for pollen-tube transmission, which is typically angiospermous. To show that the stigmatic region of the gynaecium of *Reseda* is, indeed, less isolated in structure than is sometimes supposed, we may compare it with that of *Papaver*, also a member of the Rhoeadales, but belonging to a different family. That the apical parts of these two gynaecia, despite their individual peculiarities, are



yet interpretable on corresponding lines, is demonstrated in Fig. 2. To facilitate the comparison a 4-carpelled gynaecium of *Papaver Argemone* L. has been chosen to set side by side with a 4-carpelled gynaecium of *Reseda alba* L. Marked as the divergences seem at first glance to be, they are found



Diagrams showing the structure of the top of the gynaecium seen from above. The arrows & dotted lines represent the boundaries between the carpels. Transmitting tissue, whether superficial or enclosed in canals, dotted. A, B, C, D, position of carpel midribs; A', B', C', D', carpellary margins.

FIG. 2. Highly schematized diagram of the top of the gynaecium in *Papaver Argemone* L. and *Reseda alba* L. A four-carpellary gynaecium of each has been chosen for purposes of comparison. For description see text.

to arise in the main out of a simple difference in degree in the development of the median region of the carpel. In *Papaver* (Arber, 1938) the midribs (the position of which is marked by the letters A, B, C, D, enclosed in brackets) do not reach to the apex, so that the top of the stigmatic crown is formed exclusively by the marginal regions of each carpel in a state of fusion (A'A'; B'B'; C'C'; D'D'). In correlation with this, the transmitting canals do not draw together at the apex. In *Reseda*, on the other hand, the midrib reaches almost to the extreme tip of the carpel, and the marginal transmitting strands converge towards it, finally meeting apically (Fig. 1, F).

The gynaecium of *Reseda* is one of the most familiar examples of a gynaecium with several carpels joined edge to edge, thus forming one loculus only. It has commonly been held that 'parietal' placentation of this kind represents one end of a graded series, the other end of which is formed by the completely 'axile' placentation in which the laminar wings of each carpel curve inwards to meet one another, while they are united back to back with those of adjacent carpels. This view has, however, been called in question by Troll (1928; 1935, pp. 14, 15; see also Wilson and Just, 1939, pp. 121 et seq.). Troll distinguishes sharply between 'paracarpous' gynaecia with parietal placentation and 'syncarpous' gynaecia with axile placentation; he regards this distinction, not merely as a matter of descriptive convenience, but as having a real interpretative value: 'sie nicht nur beschreibenden sondern erklärenden Charakter trägt.' He states definitely that paracarpous and syn-



carpous forms are not homologous, the fertile part of the paracarpous ovary being equivalent to the stylar segment alone of the syncarpous, while the fertile segment of the syncarpous ovary, if represented at all in the paracarpous, is an extremely reduced sterile zone at the base. Troll not only refuses to homologize paracarpous and syncarpous ovaries, but he also puts such gynaecea as those of *Nigella* into a distinct class apart from the syncarpous, since he regards their 'syncarpy' as being merely apparent and not genuine.

The basic defect of Troll's classification of gynaecea seems to be that he has taken what is, in fact, a graded series of structures, and separated them into distinct 'types' by means of artificial cleavages. Auguste de Saint-Hilaire's dictum (1838), though more than a century old, still remains indisputable: 'Quant aux placentas pariétaux', he writes, 'j'ai prouvé . . . qu'ils se nuancient, par des dégradations insensibles, avec les placentas axiles; par conséquent, ils ne peuvent appartenir à un autre système.' No one who is accustomed to looking at microtome series through ovaries can fail to be aware of the impossibility of drawing a rigid distinction between parietal and axile placentation. The existence of transitions between the two was emphasized by Goebel (1933, p. 1904), who rejected Troll's view that the fertile region of a multi-carpellary gynaeceum of one loculus is not homologous with that of a gynaeceum with several loculi. It may be suggested that to deny the homology between these two types of ovary is almost as unreasonable as to deny the identity of a group of people standing in a ring with arms outstretched and hands clasped, and the same group closed up, shoulder to shoulder, with all their clasped hands meeting in the centre.

Troll not only insists upon a clear-cut distinction between gynaecea with parietal and with axile placentation, but he is also disinclined to admit gradations between apocarpous gynaecea and those with axile placentation. *Nigella* may be taken as an example of a gynaeceum which is commonly held to show such transitional characters, but Troll (1933, p. 285) regards the union of the carpels in this case as something quite different from the union which occurs in a syncarpous gynaeceum. In *Nigella* he considers that the carpels are really free, but are cemented together, as it were, both centrally and laterally by the upgrowth of axis tissue. A slight upward prolongation of the receptacle in the central region between the carpels is, indeed, not uncommon in gynaecea of various types, and there may very well be some axial tissue—indistinguishable from the carpellary tissue—in the centre of the *Nigella* gynaeceum. But Troll's theory demands more than this, since he also assumes the existence of axial tissue forming radiating plates between the carpels. I have cut microtome series through the gynaeceum of *N. arvensis* L., and of a second species (a double garden form of *N. damascena* L.), and in neither case is it possible to detect any evidence of Troll's radiating plates of axial tissue uniting the carpels. There is nothing in the appearance of the sections to preclude the idea that the ovary is formed of carpellary tissue alone, with perhaps a small central plug of axial nature.

In comparing paracarpous gynaecia with other types Troll points to the fact that paracarpous is often associated with the absence of elongated styles (e.g. *Reseda*, Cruciferae, &c.), and he regards this as confirming his view that in these gynaecia the styler region is used up, as it were, to form the fertile ovary. It may be questioned, however, whether there is any causal connexion, such as Troll postulates, between paracarpous and absence of styles, for in the Gentianaceae, and other families, parietal placentation may be associated with a well defined style. The degree of gynaecial elongation between the ovary and stigma is probably a resultant of complex processes of relative growth; it is unlikely that it can be explained on purely morphological grounds.

#### 4. SUMMARY

1. It is shown by a study of serial sections of the apical region of the gynaecium of *Reseda alba* L. that there is a normal mechanism of transmitting canals leading from the stigmas down into the interior of the ovary above the placentas. Despite its open character, the gynaecium is thus, in its system for pollen-tube conveyance, typically 'angiospermous'.

2. In connexion with the parietal placentation (paracarpous) of *Reseda*, Troll's classification of gynaecia (1928, &c.) is considered, and it is concluded that the sharp distinctions which he draws between syncarpous, paracarpous, and apocarpous types are artificial and cannot be maintained. These different forms constitute a graded series, and transitions between them can be found even within individual gynaecia.

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The Organization of the Shoot in Heracleum in the Light of Development

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With Plate I and fifty-three Figures in the Text

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INTRODUCTION

RECENT studies of development in the angiosperm shoot seem to justify its treatment as an articulate structure, although the unit of construction, the phyton of Schüepp (1934), may be variously interpreted.

Some of the recent developmental studies have stressed the differences in the shoot organization as seen in dicotyledon and monocotyledon respectively (Priestley, Scott, and Mattinson, 1937). Thus the early tangential growth of the monocotyledon leaf primordium has been contrasted with the more localized and radial growth of the leaf primordium of the dicotyledon, particularly in view of the obvious correlation with the characteristic difference in the embryo. When the embryonic apex had completed its development in the pre-maturation growth phase in the monocotyledon, the temporary activity of the whole circumference of the apex might well result in the production of only one leaf initial, but if the whole apex had been similarly active in a dicotyledon shoot apex, where a primordium occupies a smaller proportion of the apex, at least two primordia must be present. This argument links the cotyledon number with the difference in growth in the primordia at the apex. The

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Ranunculaceae and Umbelliferae are unusual amongst dicotyledons in that the adult leaf primordium completely encircles the axis, and it is suggestive that the best established cases of monocotyledonous seedlings amongst dicotyledons are found in these families.

These considerations seemed to suggest a re-examination of shoot organization and development in these families and the result of such a study of *Heracleum* is now presented.

This developmental study needs prefacing by a brief statement of the characteristic adult morphology and anatomy, as the ensheathing leaf bases and the remarkable bud insertion associated with them need preliminary elucidation.

A brief examination of the nomenclature employed in the description of developmental phenomena has also seemed a necessary preliminary.

### MORPHOLOGY

*Heracleum Sphondylium* L. is a herb with a perennial underground root-stock, which expands and thickens considerably with years. Each well developed stock of several years' growth shows one or more deep scars at the apex, indicating the position of former inflorescence axes, and bears a number of lateral buds in varying stages of development (Pl. I, Fig. 1). The larger buds are enclosed in the sheathing bases of leaves, whilst the smaller are enclosed in scale leaves with a rudimentary lamina. The root system contributes to a storage region, whilst the axial portion of the root-stock is tuberous and shows, in the upper region, scars of old leaves with remnants of veins still persisting. There are no internodes and the successive leaf cushions (see p. 52) are in contact with each other all round the stem; each cushion is thicker on the side of the leaf insertion but completely encircles the stem. As a result of the development of the lateral buds, each plant, bearing a single terminal flowering shoot originally, gives rise to a large number of flowering shoots in the course of years.

Buds occur in the axils of nearly all the older leaves, but of these only a few develop into active shoots, the rest remaining dormant. They occur opposite the median bundles of the subtending leaves, not, however, on the axis but on the leaves themselves, and like the latter, their insertions spread tangentially round the node and the more vigorous completely encircle the axis. These girdle-like bud insertions become thick and smooth with age and are here described as bud cushions; they persist after the secondary thickening of the root-stock and lie on the outer surface of the latter like the woven strands of a basket (De Bary, 1884, pp. 309-10). At the base of the flowering shoot lies the active bud for the next season, and at a later stage the cushion of this bud forms a thick smooth rim round the deep scar left by the dead flowering shoot (Pl. I, Fig. 1). In connexion with a very active lateral bud a new tap-root may be developed, otherwise the bud vascular supply may contribute to the main tap-root.



The seedling (Text-fig. 1, a-c) has a tap-root and a very short stem which bears a pair of long narrow cotyledons and a leaf with a long petiole and a sheath which encloses the terminal bud. Though the germination is epigeous,



TEXT-FIG. 1. *Heracleum Sphondylium* seedlings. ( $\times \frac{3}{4}$ .)

the hypocotyl is little over 0.5 cm. in length, and it is mainly the elongation of the cotyledon petiole which carries the lanceolate lamina above ground. The first few leaves remain simple, reniform in outline, dentate, long-petioled, and with sheathing bases. By the time four or five leaves are unfolded, the root and hypocotyl have accumulated sufficient food to become fusiform in shape and distally a few branch roots are developed, whilst the short stem thickens a little in the middle and bears scars of former leaves. From this stage there is a transition from the reniform lamina, through a simple tri-lobed, to the compound pinnate type with three to five main segments, which are again compound and lobed. Each season the terminal bud of the seedling resumes

active growth in March and grows vegetatively for at least two seasons before it develops an inflorescence to flower the following season. The flowering shoots are maturing fruit during early July, when the leaves gradually die off and the whole shoot dries out and dies down to leave a deep scar on the stock. The more vigorous of the lateral buds on the stock grow vegetatively for about two seasons before flowering and thus repeat the cycle of the original terminal bud without severing connexion with the stock.

*The flowering shoot.* The stem is erect and stout, ribbed and dull green with numerous short stiff hairs. It usually reaches a height of 3 to 4 ft. The first few internodes are short and solid, whilst the upper are long and hollow. The biggest leaves are found at the lower nodes and the size falls off up the inflorescence axis to the bracts of the upper region in which the petiole is not developed, the lamina much reduced and the sheath the most conspicuous part. Branch inflorescences are only developed in the axils of the upper bracts.

The primary branchings of the compound umbels consist of 10 to 30 stout, almost straight, rays and the bracts, if present, are few and deciduous.

*Phyllotaxis.* Lateral buds in varying stages of development are found on the root-stock at the close of the flowering season. From serial transverse sections from well developed buds the arrangement of the leaves in the bud was drawn with the help of a micro-projector. The sixth and seventh leaves are approximately superposed above the first and second leaves respectively, indicating that the phyllotaxis is pentastichous. This is confirmed by a study of transverse sections of an internode just below the node. The angle subtended at the centre of the stem by the main leaf trace bundles of two successive leaves was found to be approximately  $142^\circ$  to  $144^\circ$ ,  $144^\circ$  being the angular divergence in a  $2/5$  spiral system.

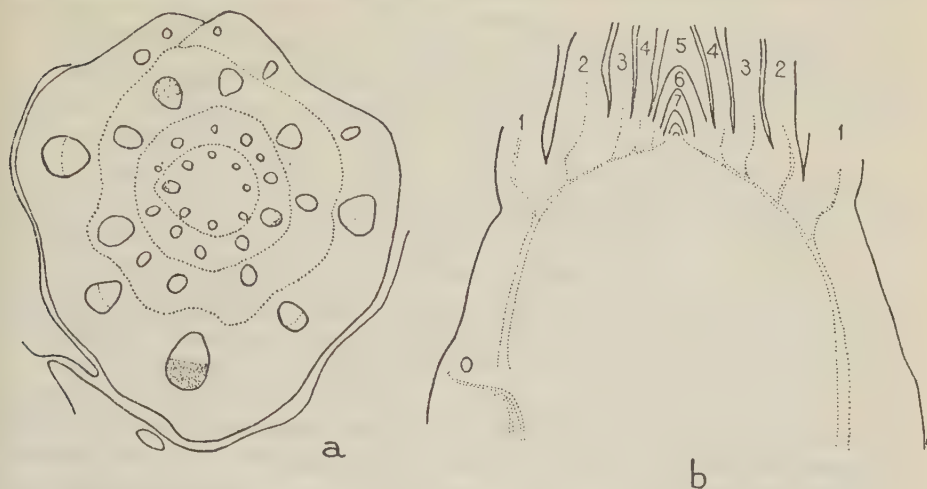
In microtome sections of the terminal bud the largest number of primordia noted was 9, but owing to certain features of the primordia in development, an exact measurement of the divergence from such sections was not readily obtained and varied from about  $158^\circ$  between the two youngest primordia to about  $148^\circ$  between the next two, so that it appears that the divergence of the typical  $2/5$  system of  $144^\circ$  is only stabilized in adult regions.

#### ANATOMY

*The vegetative axis.* The typical dicotyledon vascular ring occupies only a very short region at the extreme base of the shoot which, with the tap-root beneath, forms the root-stock of the plant. Above the root-stock the remaining short shoot of this vegetative rosette stage consists of a succession of leaves of increasing size, followed by an abruptly narrowing, broad-based cone where younger primordia are still more crowded in the centre of the bud.

The crowded succession of leaf insertions on the rapidly widening axis just below the apical cone, without any vertical extension, accounts for the fact that in cross-section of the rosette axis near the apex it is usual to find sometimes four concentric rings of bundles (Text-fig. 2 a); no linkage of bundles

occurs in the three outer rings, which are probably more correctly regarded as three overlapping leaf insertions in which the bundles from the leaf base are following an oblique downward course (Text-fig. 2 *b*) to take up their position in the innermost ring where, at a later developmental stage, the leaf traces of



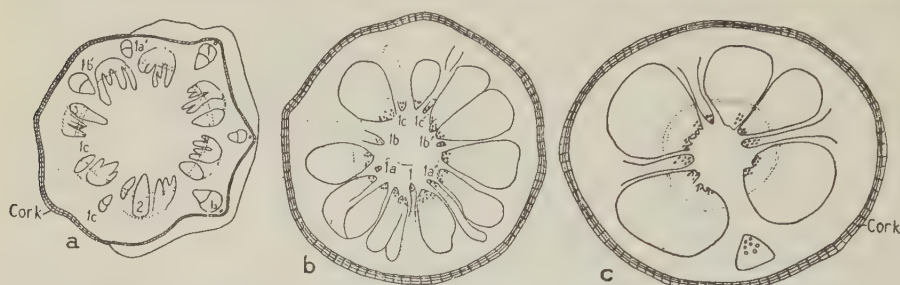
TEXT-FIGS. 2 *a* and *b*. *Heracleum*. 2 *a* transverse and 2 *b* longitudinal sections of the bud. ( $\times 10$ .)

successive leaves are associated to form the vascular system of the axis. Ultimately periderm forms across the leaf cushion, and the regions with the outer concentric rings of bundles seen in sections close beneath the apex now rot away to leave a smooth cork surface. Thus it is clear that the concentric rings of bundles seen in young regions have nothing in common with the 'scattered' arrangements of the bundles which characterize both young and adult regions of monocotyledon shoots.

In the bud of the seedling plant the leaves are arranged in a  $2/5$  clockwise, spiral phyllotaxis. The small leaves at the base usually have a trace system of five bundles and do not completely enclose the axis, but the leaves formed subsequently increase in size, extend almost completely round the axis, and have a trace composed of a large number of bundles; in a seedling with seven leaves some of the later-formed have as many as five lateral bundles to each side of the median, and still larger plants have larger leaves associated with a still more complex trace system.

Each trace bundle of a leaf has a free course through one internode and then comes to lie very close beside bundles from higher leaves with which it is later united by common cambial activity to form a synthetic bundle (Text-fig. 3 *a*). Thus a section near the base of the plant shows an alternation of isolated trace bundles of the oldest leaf with groups of bundles from higher leaves which are tending to unite to form synthetic strands (Text-fig. 3 *b*).

In older parts of the axis the periderm and a parenchymatous cortex surround the vascular ring. The bundles are clearly demarked from the cortex by the crushed and tangentially stretched caps of protophloem, which still stain deeply with fast green. The cambium in the synthetic regions remains active



TEXT-FIGS. 3 *a-c*. *Heracleum*. Transverse sections of axis at three levels to show the construction of the vascular ring of alternating leaf trace and synthetic bundles. The distribution of the phellogen activity is indicated. (3 *a* and *b*  $\times 15$ , 3 *c*  $\times 30$ .)

for a long time and the tissue formed is largely parenchymatous in nature, relatively more being formed on the phloem side. The pith in the basal region remains relatively small.

Text-fig. 3 *b* shows the change in the course of the bundles from the expanding region at the apex, where the bundles of the ring follow an almost horizontal course as they are carried out over the expanding pith to the adult parts where they run vertically. Consequently, in a cross-section of the lower adult part of the axis the bundles are cut transversely, except at their level of entry from a leaf, where they have a short horizontal course.

Text-fig. 3 *c* is cut very low in the axis and shows three strands running horizontally across the vascular ring, whilst one bundle from the same node is still in the cortex; the small number of bundles contributed at this node, the narrow pith, and the absence of other contributions of trace bundles below this level suggest that this section was cut at the cotyledonary node; sections below this level show the protoxylem of the trace bundles to be much pulled out, as would be expected during the early elongation of the hypocotyl.

As the trace bundles enter the vascular cylinder, the protoxylem runs horizontally across the widened ring to take up its position at the periphery of the pith, whilst the protophloem of the same bundles remains at the margin of the cylinder on the same periphery as the protophloem groups of the synthetic bundles; below this the protophloem and protoxylem both have a vertical course. On the radii occupied by synthetic bundles the region between protophloem and protoxylem is composed of secondary vascular tissue derived from the fascicular cambial activity, but the cambium is not active in the trace bundles close beneath their entry into the axis, nor has the cambium spread across the inter-fascicular region, so that the trace bundles are under considerable strain to maintain continuity of tissues. The cells of the vacuolated



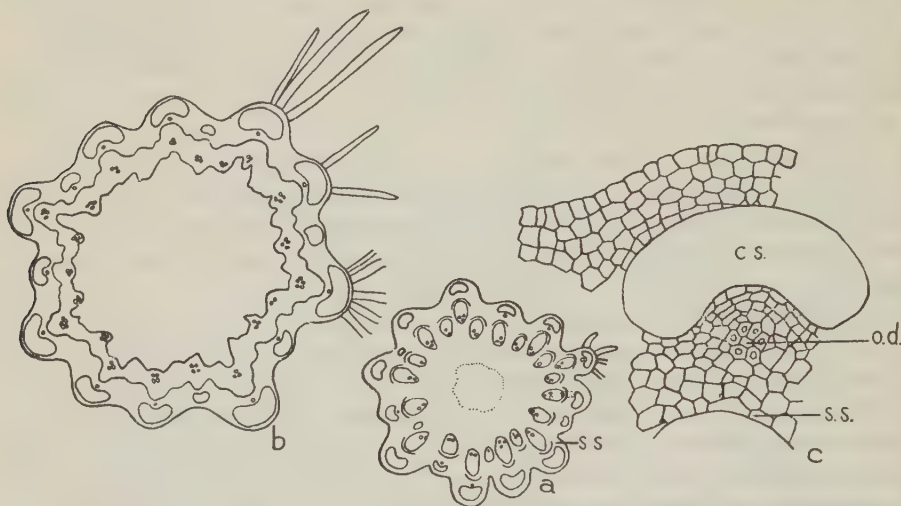
cambium of the trace bundles are stretched radially and undergo occasional tangential divisions, so that the tissue separating xylem and phloem on the radii of these bundles is similar in appearance to the ray tissue which separates them on either side from synthetic regions of the ring. The absence of active cambium in the regions of recently entered trace bundles is also shown by the pattern of cambial distribution in a longitudinal, tangential view. The active cambium in the synthetic bundles follows the course of bundles from higher leaves and is consequently forked or deflected around entering trace bundles at each node.

The cambium is always most active in the synthetic bundles, and traced downwards in the axis more and more of the bundles become linked by common cambial activity; Text-fig. 3 *a-c* shows stages in this progressive linkage. Still later the cambial activity from the synthetic bundles spreads laterally across the parenchyma in front of the trace xylem to give a complete ring of active cambium.

Cork is well developed in the region below the insertion of the sixth leaf from the apex, where the outline of the axis is almost completely circular (Text-fig. 3 *b*). The origin of the phellogen, however, is seen in the leaf cushion of the next higher leaf (Text-fig. 3 *a*). The phellogen passes immediately outside the median and lateral strands and then moves outwards to the epidermis till it meets the cork already formed in the adaxial epidermis of the sixth leaf. Thus the complete ring of phellogen is partly pericyclic and partly epidermal in its origin. In one part it runs in the cortical region immediately external to the oil duct, that is in a region below a leaf insertion where the parenchyma is continuous from the cortex of the internode into the leaf cushion (Text-fig. 3 *a*). On the adaxial side of the leaf insertion, at an early stage of development, cork can be seen to be arising in the epidermis of the leaf cushion, but this is no longer recognizable in the mature axis. This is further borne out by the fact that a little lower down in the axis, where a complete ring has been formed on one side of the axis, the cork passes immediately outside the vascular bundles, and on the other side some parenchymatous tissue intervenes between the cork and the vascular bundles. Further down in the axis, presumably in the hypocotyl, as there is no longer any indication of bundles running horizontally to leaf cushions, this arrangement is lost and the periderm is quite regular outside the ring of vascular bundles.

*The flowering axis.* In the flowering axis the ribs on the stem alternate in successive internodes and correspond with the subepidermal strands of collenchyma. The epidermis has a thick mantle of cuticle which is easily separated by maceration; it bears numerous unicellular hairs, particularly in the younger regions, and each larger hair has its rounded base embedded in a multicellular cup-shaped emergence. The cells of the epidermis show for a considerable time their origin in groups from a common mother cell, mainly by repeated anticlinal divisions at right angles to each other. Beneath the epidermis follow two or three hypodermal layers of cells with thick tangential walls and which,

in the adult stage, give the appearance of *plattencollenchym* (Müller, 1890). Normally two layers of hypodermis separate the collenchyma strands from the epidermis, but occasionally a periclinal division occurs in the inner hypodermal layer (Text-fig. 4 c).



TEXT-FIGS. 4 a-c. *Heracleum*. 4 a. Transverse section of young region of the axis with bundles isolated beneath collenchymatous ridges; starch sheath (s.s.) incomplete. 4 b. Older region of axis with bundles linked by libriform tissue. 4 c. Cortical region of a ridge to show the collenchyma strand (c.s.) and the oil-duct (o.d.). (4 a and b  $\times 18$ , 4 c  $\times 180$ .)

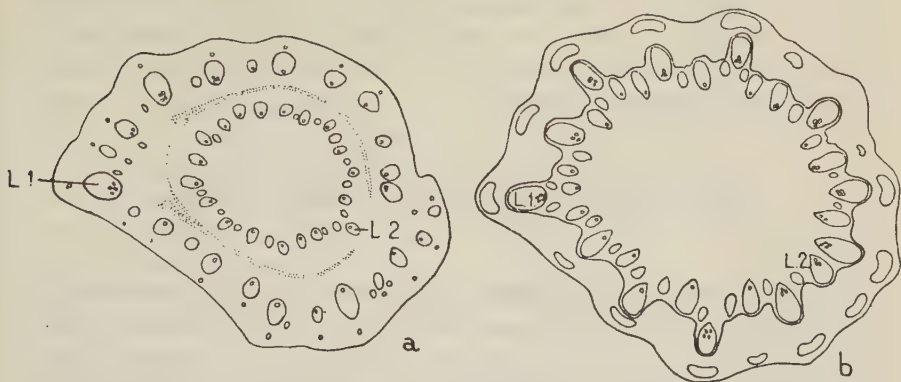
Each collenchyma strand is more or less reniform in outline and curved over an oil-duct, from which it is separated by two to four layers of parenchyma; rarely an oil-duct is seen incorporated in the collenchyma strand. The strands run a straight course down the internode, but at the base of the latter the cells become shorter, abundantly pitted, and more uniformly thickened, they become more tangentially extended round the periphery of the node, and finally lose all characteristic collenchymatous features.

The ribbed nature of the flowering axis is definitely correlated with the development of collenchyma, and this in its turn follows the course of a vascular strand down the petiole and internode and disappears when this bundle is united with others into a synthetic bundle at the node. The collenchyma strands, like their underlying trace bundles, thus alternate with those of the internodes above and below.

Within the hypodermis and collenchyma lies the general cortex which, between the primary oil-duct near the collenchyma strand and the phloem, consists of about four to six layers of parenchyma (Text-fig. 4 c). The starch sheath in the younger regions appears outside each trace bundle (Text-fig. 4 a) and only becomes continuous at a later stage (Text-fig. 4 b).

The bundles of the vascular ring differ in their structure and time of differen-

tiation. In an early stage the ring is composed of trace bundles of the leaf immediately above (situated beneath the collenchymatous ribs), which are the largest and most prominent bundles in the ring and have a number of protoxylem vessels differentiated, together with trace bundles of the next higher



TEXT-FIGS. 5 *a* and *b*. *Heracleum*. Transverse section 5 *a*. at node of insertion of L 1, 5 *b*. of internode below insertion of L 1. ( $\times 30$ .)

leaf with only one or two protoxylem vessels and lastly a small number of desmogen strands. The bundles are isolated from one another by ground tissue (Text-fig. 5 *b*).

The cambium becomes active in the foliar and trace bundles at an early stage, and secondary growth is confined to these bundles for some time. Meanwhile intense activity with irregular cell divisions is resumed in the ground parenchyma between individual bundles on about the same periphery as the xylem, giving rise to a tissue which, between the bundles, is ultimately transformed into a libriform tissue, whilst two or three cells on the flanks of the trace bundles remain as parenchymatous rays. The cambium then becomes continuous outside the libriform tissue and the xylem, with the libriform tissue, forms a continuous ring of lignified tissue (Text-fig. 4 *b*).

Oil-ducts are a very characteristic feature. They accompany each collenchyma strand and occur outside the phloem of each group of synthetic bundles, on the sides of the vascular bundles, in the secondary phloem, and in the intraxylary parenchyma. They are the first structures to differentiate in the ring of meristem in the axis and precede phloem differentiation. They run a straight course near the collenchyma strands down the petiole and internode, but anastomose and often branch in the nodal region.

Text-figs. 5 *a* and *b* illustrate the leaf insertion at a node and the structure of the internode immediately below and show how the configuration of the dorsal surface of a leaf sheath determines that of the internode below. In Text-fig. 5 *a* the vascular ring of the axis (base of the upper internode) is composed of the trace bundles of the two upper leaves L 2 and L 3 alternating with one another. Bundles of L 2 have protoxylem differentiated in them, whilst those



of L<sub>3</sub> are as yet merely desmogen strands. Text-fig. 5 *b* shows the structure of the nodal region just below the insertion of L<sub>1</sub>. The axis now shows two concentric rings of bundles, the outer belonging to L<sub>1</sub>, the inner to L<sub>2</sub> and L<sub>3</sub>. Most of the bundles of L<sub>1</sub> and L<sub>3</sub> lie almost on the same radii, whilst those of L<sub>2</sub> alternate with them. The bundles of L<sub>1</sub> in the outer ring then follow an oblique downward course and near the base of the internode come to form, with the remaining bundles, a single vascular ring of approximately double the number of bundles in L<sub>1</sub>.

## DEVELOPMENT OF THE VEGETATIVE SHOOT APEX

### *Nomenclature.*

Before attempting the description of the shoot apex of *Heracleum* it is necessary to define certain terms, as their loose employment readily leads to confusion.

*Bud.* This term refers to that portion of the shoot which includes the free apex and the region immediately below it occupied by at least one complete cycle of foliar primordia, according to the normal system of phyllotaxis in the adult plant. In *Heracleum* with a 2/5 system the bud includes the free apex and the region occupied by at least five primordia immediately below it.

*Shoot apex.* This term will refer to the free apex above the youngest leaf primordium. It may include a foliar foundation before the erection of the primordium.

The first and the most obvious distinction in the bud concerns the types of tissue present. The cells are all, in the broad sense, meristematic, but two distinct regions may be recognized:

*a. Eumeristem* (Kaplan, 1937), in which the cell contents are dense and show no obvious distinction into parietal protoplasm and vacuole. Other distinctions associated with this are a very thin wall and absence of intercellular spaces. This is equivalent to the embryonal meristem of Koch (1893, p. 526). 'Promeristem' of Eames and MacDaniels (1925) is avoided, as the tissue in question is already a meristem.

*b. Vacuolating meristem* (*Urparenchym* of Kaplan, 1937) is composed of actively dividing cells in which vacuoles are recognizable and intercellular spaces arise relatively early between the cells. The form of vacuolating meristem occurring in the axis of the bud is the file meristem (*Rippenmeristem* of Schüepp, 1926), in which the cells emerging from the eumeristem tend to elongate and to divide almost entirely by transverse walls.

*Urmeristem* of Schüepp (1926) appears to include both eu- and vacuolating meristem. *Urparenchym* of Kaplan tends to lose the emphasis on the continued activity of cell division.

The shoot apex may also be analysed in terms of histogens, based primarily



on the planes of cell division. The two sets of terms may then be compared as follows:

	Hanstein (1868).	Büder (1928), Schmidt (1924), &c.
<i>Dermatogen</i> ,	Anticlinal divisions only, in the	<i>tunica</i>
<i>Periblem</i>	regions of the apex above the	
	primordia.	
<i>Plerome</i>	Irregular planes of division.	<i>corpus</i>

Hanstein associated with his terms an implication that the dermatogen would give rise to the epidermis, the periblem to the cortex, and the plerome to the stele (Foster, 1939); this confuses the issue, as this further differentiation is not consistent in different types. Shorn of any implication as to the tissues that may be derived from the histogens, both terminologies seem equally satisfactory.

In plants in which the pith is recognizable above the last primordium a few of the central cells of the free apex are already in transition to file meristem, and the distinction between these vacuolating cells and the outer layers of the eumeristem is more marked than that between the plerome or corpus and the outer layers. This is the distinction emphasized by Koch (1893), who speaks of a sheathing tissue (*Hüllgewebe*) of embryonal meristem surrounding a central tissue in which vacuolation is proceeding. He adds the further implication that the sheathing layers will give rise to all the tissues outside the pith, while the pith alone is derived from the central tissues. The primordia are derived from the upper flanks of the sheathing tissue, whilst lower down the prodesmogen appears in the inner portion adjacent to the central tissues.

*Prodesmogen*. In cross-sections a little lower in the bud a ring or an arc of meristematic cells becomes evident owing to pith and cortical vacuolation, or the former alone. In this prodesmogen stage the cells are not appreciably elongated and thus show little difference from the eumeristem of the apex, with which this tissue, in longitudinal sections, is seen to be in continuity. *Desmogen* consists of elongated meristematic cells, which give rise to vascular elements. Helm (1931 and 1937) has suggested that primary meristem would be a preferable term to prodesmogen, since this ring of meristem will ultimately give rise to both vascular tissues and rays. In *Heracleum* it is certainly difficult to apply any of these terms to the earlier stages of differentiation in the bud. When a region of meristematic cells becomes isolated by central and peripheral vacuolation, the cells elongate but do not at first comply with the requirements of desmogen, as they will contribute not only to vascular strands and rays, but also to the formation of a zone of parenchyma external to the phloem, to the oil-duct, and to the inner part of the cortical collenchyma strand; thus it is not until a preliminary differentiation has taken place that true desmogen strands are identified. This difficulty in the use of the term prodesmogen also occurs in descriptions of the gymnosperm shoot apex where the cortical vacuolation is late to appear (Louis, 1935).

*Primary* and *secondary* are terms which are used in very varied senses;

they are here given a definite significance. Primary tissues are derived from the apical meristematic tissues by continuance of their original manner of division, or by conversion of these tissues into permanent tissues through an intermediate vacuolating meristem stage. Since the divisions are primarily anticlinal or irregular in the eumeristem, or transverse in the file meristem, they do not give rise to series of radially seriated cells. *Secondary* tissues increase in girth by the repeated tangential divisions of meristematic (cambium) cells, thus giving rise to radially seriated cells.

This use of the terms has the advantage that the tissues may usually be judged by a definite criterion, at least in dicotyledons. Eames and MacDaniels (1925, p. 41) speak of primary meristem as derived from promeristem and regard any meristem in continuity with the promeristem as primary (even cambium with some exceptions), and as secondary those meristems which arise by a return to the meristematic condition of already vacuolated cells (origin of phellogen). This method of use of the terms gives no clarity of definition, as is admitted by Eames and MacDaniels, who speak in general of tissues derived from a cambium as secondary tissues (1925, loc. cit., p. 130).

*Foliar foundation.* This is a translation of the term *soubassement foliaire* suggested by Grégoire (1935, 1935 *a*) and elaborated by Louis (1935). It signifies the transverse expansion of the apex above the last primordium to form a basis upon which the free portion of the next younger primordium will arise.

*Flank meristem.* More detailed analysis of the eumeristem tends to show that it usually consists of a group of central initial cells over the actual summit of the apical cone and the lateral flank meristem down the sides of the cone. In the central region the meristem cells have characteristics suggesting that they are less actively meristematic than those of the flank meristem, and whilst the former constitute the self-perpetuating meristem of the apex, the flank meristem contributes to leaf formation.

#### *The shoot apex and origin of a new primordium.*

The shoot apex of a vegetative bud is relatively low and broad; two apices measured respectively  $32\ \mu$  high by  $60\ \mu$  wide at the base and  $80\ \mu$  high by  $170\ \mu$  wide above the youngest primordium; transverse measurements are approximate owing to the asymmetry of the apex. The apex does not vary much in degree of convexity, and above the primordia is composed entirely of eumeristem (Pl. I, Figs. 2 and 3). The outer three layers are typical tunica in which the cells divide by anticlinal walls only, even at the origin of new primordia. Within these are five or six further layers of eumeristem in which, especially to either side of the actual summit, occasional periclinal divisions occur. Within these eight or nine eumeristem layers three or four cells over the actual summit gradually become converted into file meristem, so that the occasional periclinal divisions to either side of the summit merely maintain the depth of the eumeristem. As such periclinal divisions do occur the inner five or six eumeristem layers are strictly speaking corpus, but on the flanks

these layers divide entirely by anticlinal walls (except at the origin of primordia) and there does not seem to be sufficient justification for separating the inner layers into a different category from the outer three. The eumeristem in *Heracleum* is exceptionally deep, as the majority of dicotyledons appear to have a depth of not more than four to six cells.



TEXT-FIGS. 6a and b. *Heracleum*. 6a. Diagram of the shoot apex shown in Pl. I, Fig. 3, to show distribution of central initial cells (*c.i.*), flank meristem (*f.m.*), vacuolating file meristem (*v.m.*), and desmogen (*d.*). ( $\times 200$ .) 6b. The bud shown in Pl. I, Fig. 2, to illustrate the continuity of desmogen and meristem of the primordium. ( $\times 45$ .)

In the eumeristem the central initial cells (*c.i.*, Text-fig. 6a) occupy the median region of the apex in the form of an inverted cone with its broad base formed by the summit of the apex and its blunt top by the group of three or four cells of the innermost eumeristem layer which are about to be converted into file meristem (*v.m.*, Text-fig. 6a). The central initial cells are larger than those elsewhere in the eumeristem, the protoplasts appear finely vacuolate, and the nuclei seldom show mitotic figures. In superficial sections which remain in the same tunica layer, the thin-walled cells are seen to fall into groups of common origin, bounded by thicker deposits of wall material and especially thickened at the corners of the cell groups; such thickenings appear to be a feature of this region of eumeristem. This region corresponds with the zone of central mother cells described by Foster (1938) for *Ginkgo* and also appears similar to Schüëpp's apical initial group seen in some plants with apical cell growth (1926, p. 45). Foster regards the presence of such a zone of cells as a unique feature of *Ginkgo*; he also pointed out the thickenings at the corners and sides of many of the cells, a feature already mentioned by Priestley (1928) as occurring in apical meristem.

The flank meristem (*f.m.*, Text-fig. 6a) surrounds the cone of central initial cells as a ring of tissue, broadest at the free surface of the apex and narrowest where it abuts on to the cells transitional to file meristem (*v.m.*, Text-fig. 6a). The cells are more regular than those of the central initial group in shape and arrangement, the protoplasts are less vacuolated and the nuclei are more frequently seen in division. The flank meristem appears to be equivalent to the peripheral layers of Foster (1938). There does not seem to be any part of the apex which conforms strictly to Schwarz's *phyllogen* (1927), which was defined as the layer or layers of the apex which undergo periclinal divisions only in the



plane of maximal area and only at the time of leaf initiation, and which are responsible for the upfold of the meristem to form the primordium.

The upper region of the flank meristem is concerned with the initiation of primordia, but if these layers are followed downwards it is found that near the base of the first primordium late in the first plastochrone, or near the insertion of the second primordium early in the second plastochrone, they contribute to the file meristem on the inner side and also give rise to the prodesmogen. This agrees with Koch's statements (1893).

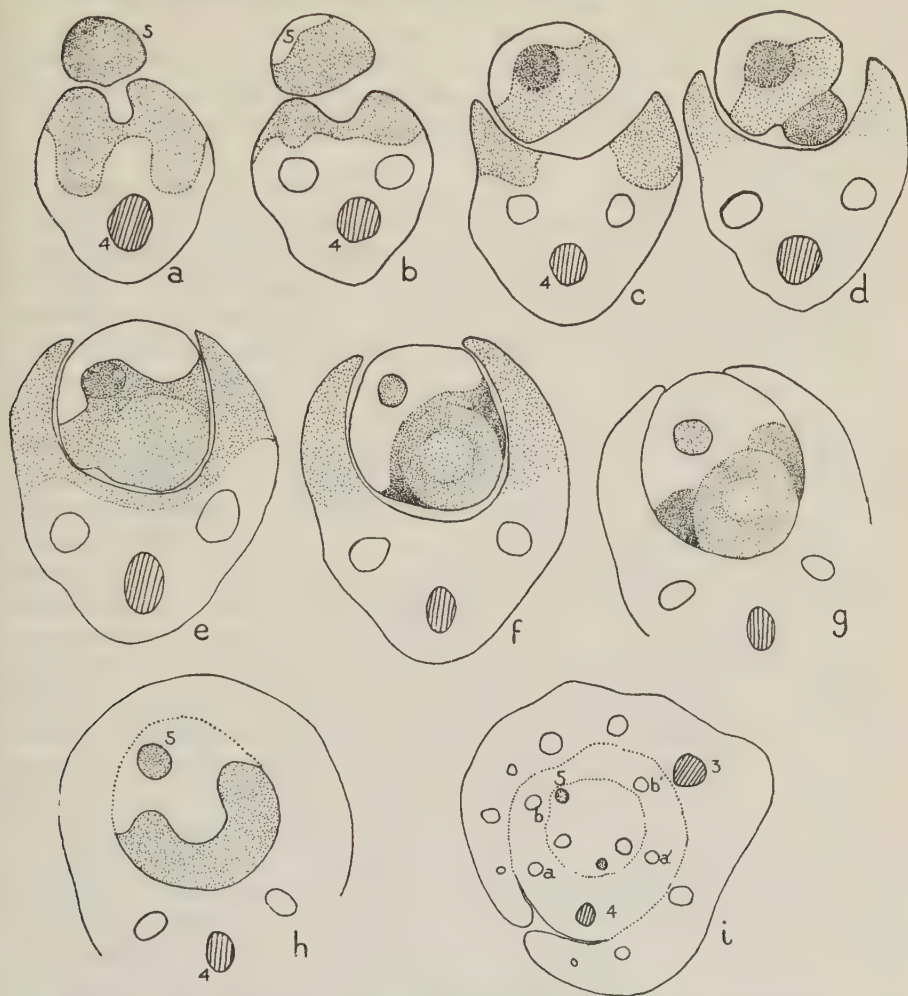
The *file meristem* should not, strictly speaking, be included in the apical meristem, as it does not appear in the vegetative apex of *Heracleum* above the last primordium. The origin of the highest file meristem from the group of central initial cells is comparable with the type of origin described by Foster for *Ginkgo* (1938) and *Cycas* (1939). In the uppermost region of the file meristem the cells divide by transverse and oblique walls, lower down by transverse walls mainly, though some longitudinal and oblique walls occur as well (Pl. I, Fig. 3).

The flank meristem is more actively meristematic than the central initial region and also, at any particular interval of time, it is found that a particular sector is more intensely active than the remaining periphery. This localized activity leads to asymmetric growth of the apex; at first no appreciable increase in depth of the flank meristem itself is evident, but the inner cells become converted into vacuolating meristem, so that the active flank region is carried outwards on a buttress of vacuolating meristem. This asymmetry on one sector is the first definite indication that a new primordium will soon appear on the apex in this region and the asymmetric growth forms the radial axial extension over which the free portion of the primordium will be erected. In serial transverse sections or where a longitudinal section passes through a region of the flank meristem where a new foliar foundation is in process of initiation, it is found that the highest prodesmogen strand recognizable in the shoot axis runs up and merges into the meristem in the active sector of the flank meristem (Pl. I, Fig. 2). This fact suggests that the special activity of successive regions of the flank meristem is caused by special nutrition from the developing vascular system below; it is seen that the prodesmogen strands differentiate up from below and later run into the free portion of the primordium as it is erected. If this conception is correct it is clearly only logical to consider the further growth at the apex in the light of the developing vascular system in the lower part of the axis.

*Vacuolation behind the apex.* As the progress of vacuolation is followed in the apex by serial transverse sections (Text-fig. 7), or in longitudinal sections (Pl. I, Figs. 2 and 3), it is found that the vacuolation which extends highest into the bud is definitely associated with the youngest primordium. This vacuolation commences in the abaxial region of the foliar foundation and gradually works up into the primordium as it is erected; at first this is confined to the abaxial region and the strand in the primordium is still continuous with



the eumeristem of the apex (Pl. I, Fig. 2), but shortly after this stage the vacuolation penetrates deeper near the median regions of the primordium and isolates the median strand as shown in Text-fig. 7*e,f*. It is, however, very



TEXT-FIGS. 7*a-i*. *Heracleum*. Serial transverse sections through the bud 7*a-g* show the stages of vacuolation to isolate the median strand of the primordium, 7*h* to develop rays and the median leaf gap, and 7*i* the differentiation of additional strands lower in the bud (*i*). ( $\times 82$ .)

striking in *Heracleum* that even at this stage the vacuolation in the primordium is quite independent from, and extends higher than, any marked differentiation of the pith, from which the vacuolated tissue in the primordium is still separated by the eumeristem of the apical cone and the adaxial surface of the primordium (Text-fig. 7*f*). Slightly later in time (or shown equally well

slightly lower in the apex as at Text-fig. 7*h*), the pith vacuolates and at the same time the vacuolation in the primordium extends still deeper and becomes continuous with the pith in the region of the median leaf gap, whilst the rest of the axis at this level is occupied by an arc of meristem which will be continuous with the meristem of the apical cone.

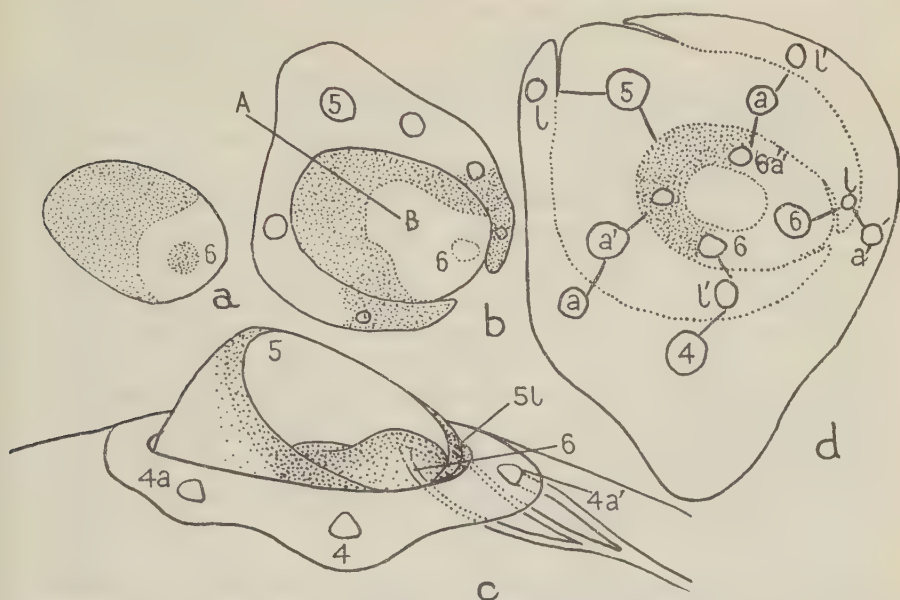
By this method of vacuolation it is evident that at the level where pith is to be recognized the residual meristem can only be present as an arc, and at no stage is it possible to recognize a ring of meristem as described by Helm and others for other plants.

The condition illustrated in Text-fig. 7*f* is developed very early in the foliar foundation of a young primordium, and it is evident that the vacuolation on either side of the median leaf trace bundle, which now becomes continuous with the pith (Text-fig. 7*h*), represents the development of the first primary rays in the axis; when the median strand enters the primordium, this vacuolation also becomes continuous with the leaf gap, but this is not yet appreciably formed in connexion with the youngest primordium 5 in the series illustrated (Text-fig. 7). Slightly later in the plastochrone vacuolation would have proceeded farther above the departure of part of the procambial ring into the primordium when the leaf gap would be more evident. It would thus appear that even in a multilacunar type like *Heracleum*, in the earliest stages of development, the younger primordium may have only the median strand differentiated and the apical meristem may be interrupted by only a single gap. The more marginal regions of primordium 5 will be derived through the extension of similar stages of differentiation into the residual arc of meristem (Text-fig. 7*h* and *i*), the stages appearing in a basifugal direction in the order: asymmetric growth, abaxial vacuolation, inward extension of vacuolation isolating the successive lateral prodesmogen strands by additional primary rays and, on entry of the strand into the primordium, forming the lateral gaps. In many types with a leaf trace of many bundles, the multilacunar stage may be preceded in development by a monolacunar stage, but this is particularly clearly seen in *Heracleum*, as there is an appreciable difference in the time of differentiation of the median and the more lateral strands and their associated gaps. As vacuolation proceeds upwards it is natural that the strands should also make their appearance in this basifugal direction. The isolation of the prodesmogen strand extends upwards through the foliar foundation and the free primordium, but in the distal region the strand becomes confluent with the apical and marginal meristem of the primordium, and it would therefore seem true to say that in *Heracleum* the prodesmogen strands are derived directly from the apical meristem and do not arise by return of vacuolating cells to the meristematic condition.

#### *The origin of the leaf trace system.*

When the youngest primordium is still a relatively narrow structure, the median strand is isolated in it by the processes of vacuolation described and

its margins continue to develop from the residual arc of meristem of the shoot apex. At this same stage of the bud primordium 5 (the second from the apex in Text-fig. 8*b*) still only partly surrounds the shoot apex, and its marginal regions are being extended by the activities of the meristematic region con-

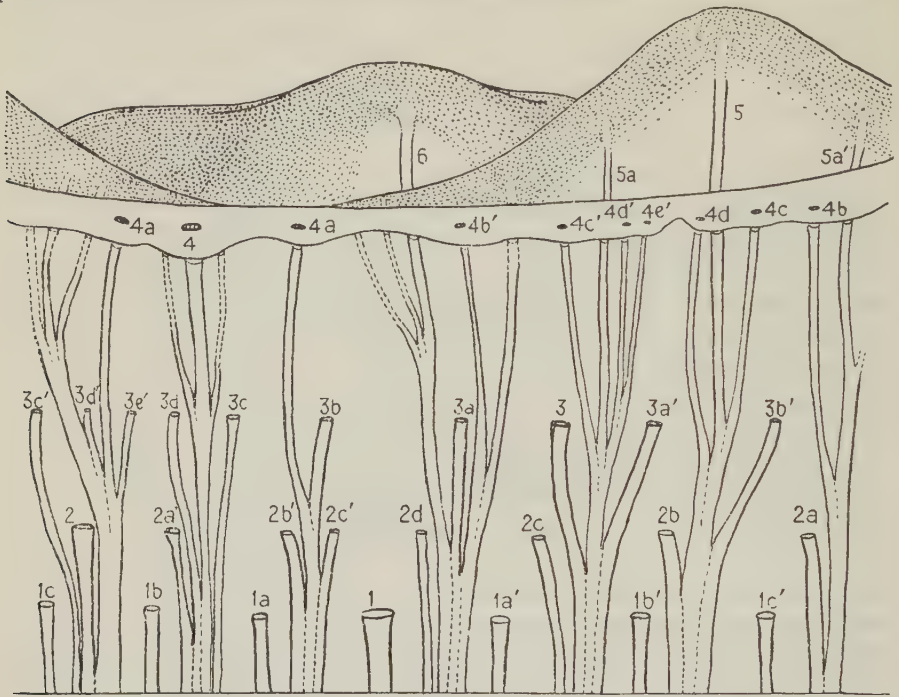


TEXT-FIGS. 8*a-d*. *Heracleum*. Diagrams to illustrate bundle linkages. 8*a*. Transverse section of bud with median strand differentiated to youngest primordium 6. 8*b*. Transverse section at lower level of bud with pith developed, L<sub>5</sub> is joining the axis, and the marginal meristem of this primordium abuts upon the meristematic region of the apex. 8*c*. Reconstruction of the bud as viewed in the plane A-B, to indicate the origin of strands from common meristem and their union into a single synthetic strand lower in the axis. 8*d*. Diagrammatic representation of bundle linkages as viewed from above.

tinuous also with the residual arc in the shoot apex. From this common meristem the more marginal strands of 5 and the laterals of 6 will be isolated by further vacuolation; strands derived in this way from common meristem and from the same sector of the shoot apex are found to be associated into a common synthetic strand lower in the shoot.

From serial transverse sections of the shoot the system of bundle linkages may be followed and represented in the form of a flat diagram (Text-fig. 9). Owing to the increasing size of leaves and the associated increase in number of bundles, such a diagram is not constructed on an exact repeating pattern for each leaf trace, but the general system of construction is evident; at the basal node the alternation of the entering leaf trace bundles of leaf 1 and synthetic bundles may be compared with that shown in transverse section in Text-fig. 3. The continuity of the strands of 5 and 6 into meristem in the bud is indicated at a stage comparable with Text-fig. 8.

Text-fig. 8c is constructed to represent in the solid the apex at the stages figured in transverse view in Text-figs. 8a and b. The median strand of the very young primordium 6 is continuous with meristem at the tip of the primordium, whilst surrounded in its lower part by vacuolating tissue con-



TEXT-FIG. 9. *Heracleum*. Flat diagram to illustrate bundle linkages.

tinuous with the pith. By the processes of tangential vacuolation followed by radial vacuolation, from this same sector of the shoot apex have successively been isolated the lateral bundles 4a' and 5b', and finally the median of 6, and these are consequently linked together lower in the shoot; at a slightly earlier stage in the plastochrone this common bundle was delimited between abaxial vacuolation and the vacuolating meristem of the future pith. It is possibly this isolation of the strand which directs nutritive materials to a particular part of the flank meristem above, the more active growth of which produces apical asymmetry and later, as the strand becomes still more localized, forms a mass of tissue by active divisions in the corpus (covered by the three-layered tunica) just ahead of the differentiating strand; this results in the heaping up of the eumeristem as primordium 6 (Pl. I, Figs. 2 and 3).

At this stage it would appear that leaf 4 was in contact with the region of the apical cone initiating leaf 6, but examination of later stages makes it clear that from the meristem of the apical cone must also be derived the marginal extensions of leaf 5, which will later surround the axis between leaf 4 and leaf 6.



Just as the median strand of 6 and second laterals of 5 are to be derived from a common region of the meristematic arc above a synthetic region of the vascular ring, so also the common flank meristem must contribute, in its distal and inner region, to the foundation above which the median part of 6 will be erected, and, in its basal and peripheral region, to the marginal part of the foundation of 5; it is very natural that these regions with different destinies should at this early stage grow as a common meristem, since they are also overlying a common meristematic strand. In the next stage tangential vacuolation appears in the mass of meristem and thus separates an inner strand which runs up in continuity with the apical meristem of the developing primordium 6, from an outer arc of meristem at a lower level. It is possible that the lower ring of meristem, supplied from below by *5b* and *4a*, forms first the extension of the foliar foundation and then the erection of the marginal regions of 5. This method of development of the median region of one primordium and the marginal regions of the next older from a common zone of flank meristem makes it more difficult to give a clear-cut limit for the foliar foundations of two successive leaves, the two foundations are initially fused, the younger foundation being enclosed and fused with the next older; and it is this manner of development which gives the shoot the construction of a series of encircling and confluent 'leaf cushions', the vascular strands of which appear in a series of concentric rings surrounding the true ring of the stele.

The term *leaf cushion* is used to designate that region of the unit in which the leaf has joined the stem but the strands have not yet taken up their position in the vascular ring of the axis. This is actually equivalent to the region of leaf insertion.

#### *Formation of the axillary bud.*

Buds occur in the axils of all the adult leaves, so that several stages may be followed in serial transverse sections of the same seedling (Text-fig. 10). The first appearance of activity is noticed in relation to primordium 4 in this figure—the third or fourth from the apex—and in this and succeeding stages the activity is clearly confined to the side of the union next the primordium; above this point the cells in the axils of the primordia do not appear more meristematic than elsewhere, so that the bud development is first indicated by vacuolating cells becoming more meristematic again and resuming active division, as described by Koch (1893).

A study of early stages of bud development emphasizes three points:

- i. The bud arises in previously vacuolated tissue. This is very clear in the earliest stages as the adaxial epidermis of the subtending primordia consists of empty-looking, much vacuolated cells, whilst the abaxial epidermis of the next inner *soubassement* consists of smaller cells with denser contents containing numerous small starch grains. These were seen in microtomed and stained sections, so that it is not possible to say whether they would have

given a direct starch reaction or whether they are more like the masked starch which has been observed as a temporary stage in the earliest vacuolating meristem in many apices.

ii. The activity commences in the superficial layer of the primordium and

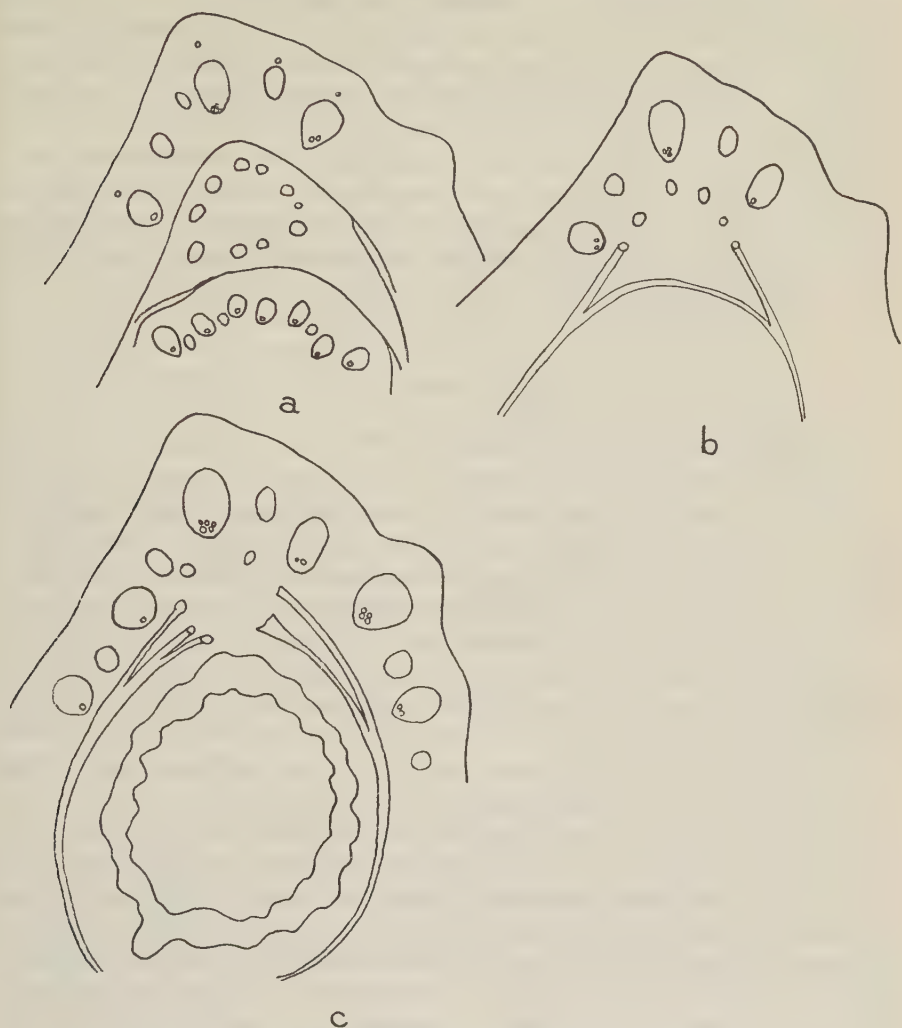


TEXT-FIG. 10. *Heracleum*. Transverse section to illustrate stages in bud development in connexion with primordia 2, 3, and 4. ( $\times 100$ .)

gradually extends from this point into the deeper layers of the leaf and leaf cushion, much in the same manner as described for *Syringa* by Koch (1893), except that in this latter type the activity is spreading in axial tissue. Serial sections show that the development is basipetal, and finally the bud strand differentiates back into the axis to unite with the prodesmogen strands which later become the synthetic strands bounding the median leaf gap.

iii. The bud activity has the unusual feature of being entirely foliar in origin. The whole development of the bud is entirely in the tissue of the leaf or leaf cushion, except eventually for the basipetal differentiation of the bud trace across the cortex to connect with the vascular ring in the axis. Louis (1935) figures the axillary bud of *Ranunculus repens* as confluent in the same way with the subtending leaf, but he states that in this type the meristem and the bud trace are initially meristematic and do not redifferentiate from

vacuolating cells. In *Heracleum* the meristematic activity in connexion with the formation of a bud not only spreads into the deeper layers towards the median bundle of the subtending leaf but also spreads laterally, right and left, along the line of union between the latter and the abaxial surface of the inner



TEXT-FIGS. 11 *a-c*. *Heracleum*. Transverse sections to illustrate stages in the insertion of the girdling bud trace. ( $\times 84$ .)

*soubassement*, which may become completely encircled. Food for this resumption of secondary activity may be provided by material (starch) present in the cells of the abaxial epidermis of the foliar foundation encircled.

Text-fig. 11 *a* shows the central region of the base of the bud with about ten desmogen strands arranged in a ring around the pith. Text-figs. 11 *b* and

c show the strands joining into two lateral groups and passing right and left into the tissue of the encircling leaf cushion almost horizontally like a girdle. From this vascular girdle pairs of branches are given off at intervals in connexion with the incoming trace bundles of the subtending leaf. Each pair bends sharply near the point of separation from the girdle and 'bestrides' the corresponding trace bundle in the leaf cushion before it joins the vascular ring of the main axis. This mode of connexion has been described by de Bary (1884) for *Foeniculum*, species of *Heracleum*, *Chaerophyllum*, *Myrrhis*, and *Archangelica*.

The connexion of the axillary bud is, therefore, made with the leaf trace bundles in the leaf cushion outside the vascular ring of the main axis, a fact quite in conformity with the foliar origin of the axillary bud. The continuity of the pith in the two systems, main axis and axillary branch, is maintained only through the medullary rays, as already pointed out by de Bary.

## DISCUSSION

### *The shoot apex and the development of primordia.*

The fact that the leaf primordium in the angiosperm invariably arises on the flank of an apex and then rapidly overtops it, is prima-facie evidence that the meristem of the shoot apex is growing more rapidly on the flanks than at the summit, but this point is not mentioned in descriptions of the angiosperm apex, and Foster (1938) in describing a similar condition in *Ginkgo*, refers to it as unique amongst vascular plants. Observations of Stubbs (1938) on dicotyledons and Sharman (1938) on monocotyledons on the contrary suggest that this condition, now placed on record for *Heracleum*, is very general in the angiosperm.

Two processes may be recognized at work in the tissue differentiation at the apex which contribute to the initiation of a new leaf primordium. (1) An increase in volume of the underlying central cells which is largely due to an increase in vacuolar space and represents the first stage in a process of parenchymatization, which will terminate in the differentiation of the ground tissue of the axis; at this stage the increase in cell volume occurs in all directions, but predominantly parallel to the long axis of the shoot and associated with cell divisions that are usually transverse. This is the initiation of the *Rippenmeristem* of Schüepp (1926) (the rib meristem of Foster (1938); the file meristem of the present account). (2) An increase in mass of the flank meristem due to the rapid growth in mass and multiplication of these cells.

Both these processes occur in an asymmetric manner in the apex, so that they produce an expansion, largely transverse at first, which is greater on one flank than elsewhere, and thus the site of a new primordium is being determined usually before there is any erection of the future primordium apex. This is the stage in the production of a leaf primordium described by Grégoire



(1935 a) and his collaborators (Louis, 1935) as the *soubassement* (the foliar foundation).

In some cases the erection of the new primordium follows so rapidly that the adaxial vacuolation in the new primordium seems to initiate the differentiation of the future pith. In many cases now, however, notably in the majority of the gymnosperms and in many small-leaved dicotyledons (Kaplan, 1936 and 1937), the expansion of the central cells is clearly independent of the previous emergence of any leaf primordium, so that it is probably safe to regard the early expansion of the cells just below the apex as contributing to the organization of a foliar foundation, rather than as a consequence following upon its formation.

As soon as the erection of a primordium begins, its subsequent growth and differentiation is so much more rapid than that of the apex which it flanks that the further differentiation of the axis below the level of its insertion is likely to be influenced much more by the primordium than by the apex. This differentiation includes that of a prodesmogen system common to leaf and axis, and in previous papers from this Department the characteristic anatomy of dicotyledon and monocotyledon has been reviewed from the standpoint of such developmental studies. The interest of *Heracleum* in this connexion is that it is a dicotyledon with the encircling leaf insertion characteristic normally of the monocotyledon. The rapid tangential expansion of the original primordium is associated with the production of the characteristic multilacunar gap and it is clear that there are differences in the organization of such a shoot axis when compared with the more typical dicotyledon where a single trace system only occupies a limited portion of the periphery of the axis. This point must be examined first, and then it may be possible to elucidate the fact that none the less the tangentially expanded leaf-trace system associated with the encircling leaf insertion links into a typical dicotyledon stem structure.

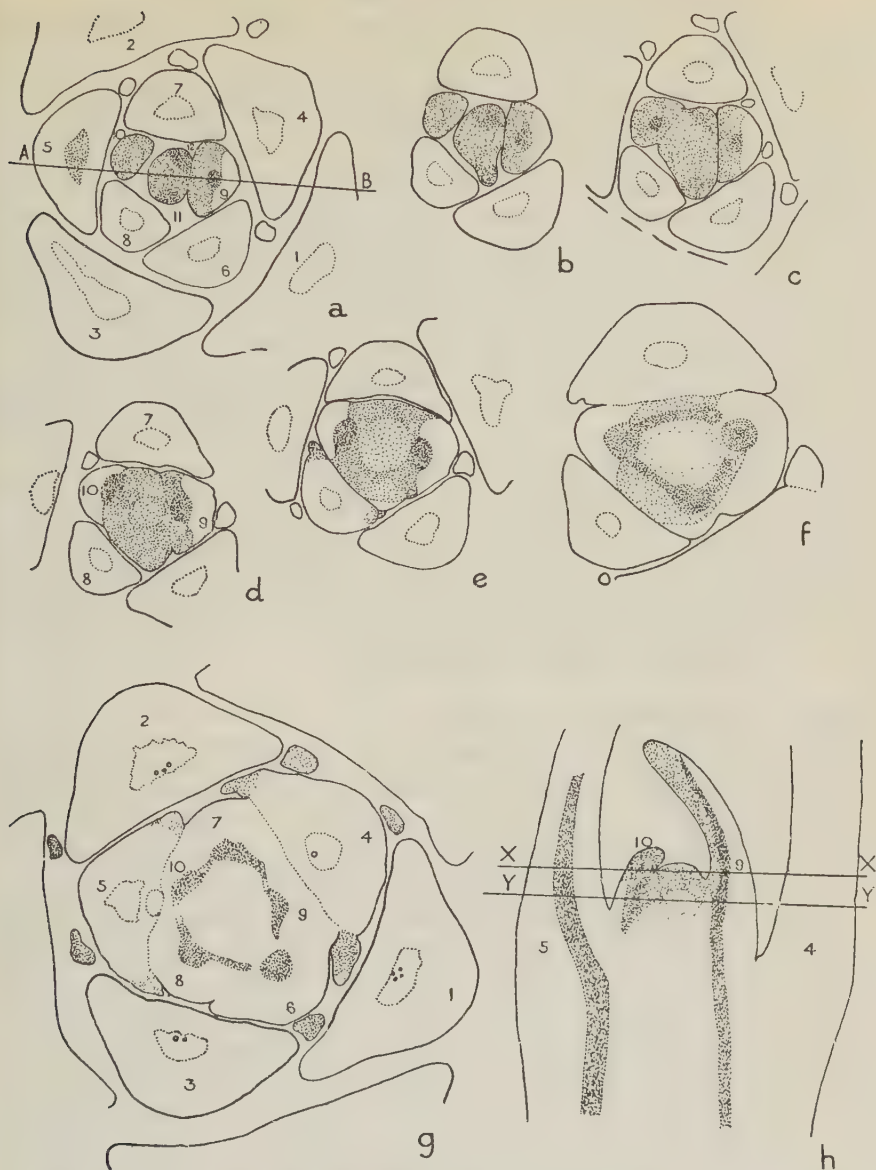
As the prodesmogen strand is isolated by spread of vacuolation from the axis into the abaxial side of the primordium, there is usually isolated between future cortex and pith a cylinder of isodiametric or slightly elongated cells, the primary meristem of Helm (1931), the procambium of other writers, the prodesmogen of this account; but in *Heracleum*, before the cylinder can be recognized, it is already interrupted by further differentiation into prodesmogen strands and rays in connexion with the tangential expansion of the youngest primordium.

In any dicotyledon where this meristematic cylinder is visible below the apex it is seen, in accordance with the asymmetric lateral expansion of the apex, to be undergoing most rapid increase where it lies beneath the newly developing primordium. Consequently, recent statements (Helm, 1931, p. 140; László Gráf, 1938) have claimed that its development proceeds under basipetal influences received from the primordium above, but this by no means follows. It is true that, later, xylem differentiation and cambial

activity usually commence at the level of insertion of the primordium and progress from thence distally into the primordium and basipetally into the axis. But in the meristematic phase of growth there is every reason to assume that we are dealing with a process taking place under strong basifugal impulses.

In the first place, the process of vascular differentiation which follows most rapidly upon the prodesmogen phase of growth is that of the protophloem, and this has been reported by practically every observer as taking place basifugally into apex and primordium in continuity with earlier differentiated phloem below; this observation has again been made in *Heracleum*. Furthermore, any angiosperm apex in the vegetative phase clearly shows progressive increase in growth activity under the impulse of the greater supplies of food rendered available by the increasing leaf area associated with continuing vegetative growth. The whole progressive increase in shoot organization thus witnesses to a general habit of growth which requires that the meristematic apex should be elaborating its organization under developmental impulses which reach it from below.

If the further development of the complete, or interrupted, prodesmogen ring be examined in the light of the fact that it will certainly be making more growth in one region than another, it is possible to see how the further development, if it leads to the formation of a primordium, must also lead to the formation of a leaf gap. Thus in *Iberis amara* (Text-fig. 12) it is seen that at first the increased apical growth is mainly transverse and the apex simply shows an asymmetric expansion, commencing at one point and restricted to one flank. Then there follows a rapid growth increase in mass of a new foliar primordium which rapidly overtops the apex (Text-fig. 12*h*); this growth of the primordium is mainly associated with growth and transverse expansion of the prodesmogen, so that at a lower level in the axis the asymmetry of the ring will correspond with such a meristematic primordium at the apex (Text-fig. 12 in *a* and *c*; compare the positions of primordia 10 and 9 with the positions of local thickenings of the ring in the axis in Text-fig. 12*g*). Comparing the series of transverse views of a  $3/8$  bud shown in Text-fig. 12 with the longitudinal view (Text-fig. 12*h*) constructed from these in the plane *A-B* of Text-fig. 12*a*, at level *X* primordium 9 is seen above its insertion and showing an indication of its median strand still associated with adaxial meristem; if this strand is followed down to a level where the meristem ring in the axis is becoming recognizable (Text-fig. 12*h* at *Y* and Text-fig. 12*f*), it is seen that the diversion of the strand into the primordium will interrupt the continuity of the ring, so that the apex growing up beyond the insertion of 9 will have a gap in the ring on this side. The same point is seen more clearly in connexion with the older primordium 5 (Text-figs. 12*h* and *g*). The leaf gap is thus no result of the basipetal influence travelling downwards from the leaf primordium, but of the basifugal impulse to more vigorous and earlier growth activity which has isolated the most vigorously growing sector of the prodesmogen cylinder in a new leaf primordium.



TEXT-FIGS. 12 a-h. Transverse sections of the bud of *Iberis amara*. 12 a-g to show the position of meristematic primordia vertically above thicker regions of the prodesmogen ring. 12 h. Reconstruction of the median longitudinal view in the plane A-B.

As the apex continues its growth, there is the usual tendency for the growth of the prodesmogen ring to increase. On the side remote from the last new primordium this will soon produce the bulge in the expanding cylinder associated with a new foliar foundation. On the other flank this increased



growth will rapidly encroach upon the leaf gap left by the departure of the arc of prodesmogen into the new primordium.

Thus the leaf gap is soon closed above, and the course of the prodesmogen on the flank of the gap, followed longitudinally, is seen as two margins encroaching over the gap until they meet above it. The sloping course of the desmogen strands in this region will be responsible for the subsequent course of differentiation of vascular elements which, running vertically in the internode above, are seen to diverge and pass to either side of the gap and the trace strand that subtends it. Thus the course of the 'reparatory' strands of Hovelâcque (1888) is determined and strands are differentiated which would easily be misinterpreted as having a purely axial course, i.e. as 'cauline' bundles (de Bary, 1884, p. 233). But in the typical dicotyledon, although the desmogen differentiation, which thus determines the course, is a basifugal differentiation of elements forward into the axis, their subsequent increase in growth will be associated with the development of new primordia and any vascular differentiation proceeding in such strands will begin at the base of a new primordium and then proceed basipetally until the original course of the desmogen strand around the leaf gap causes the process of vascular differentiation to follow the same path. Thus a reparatory (or synthetic (Priestley and Scott, 1936)) strand will be differentiating, which is only the downward continuation of a leaf trace bundle at a higher level.

#### *The leaf-trace system and the growth unit.*

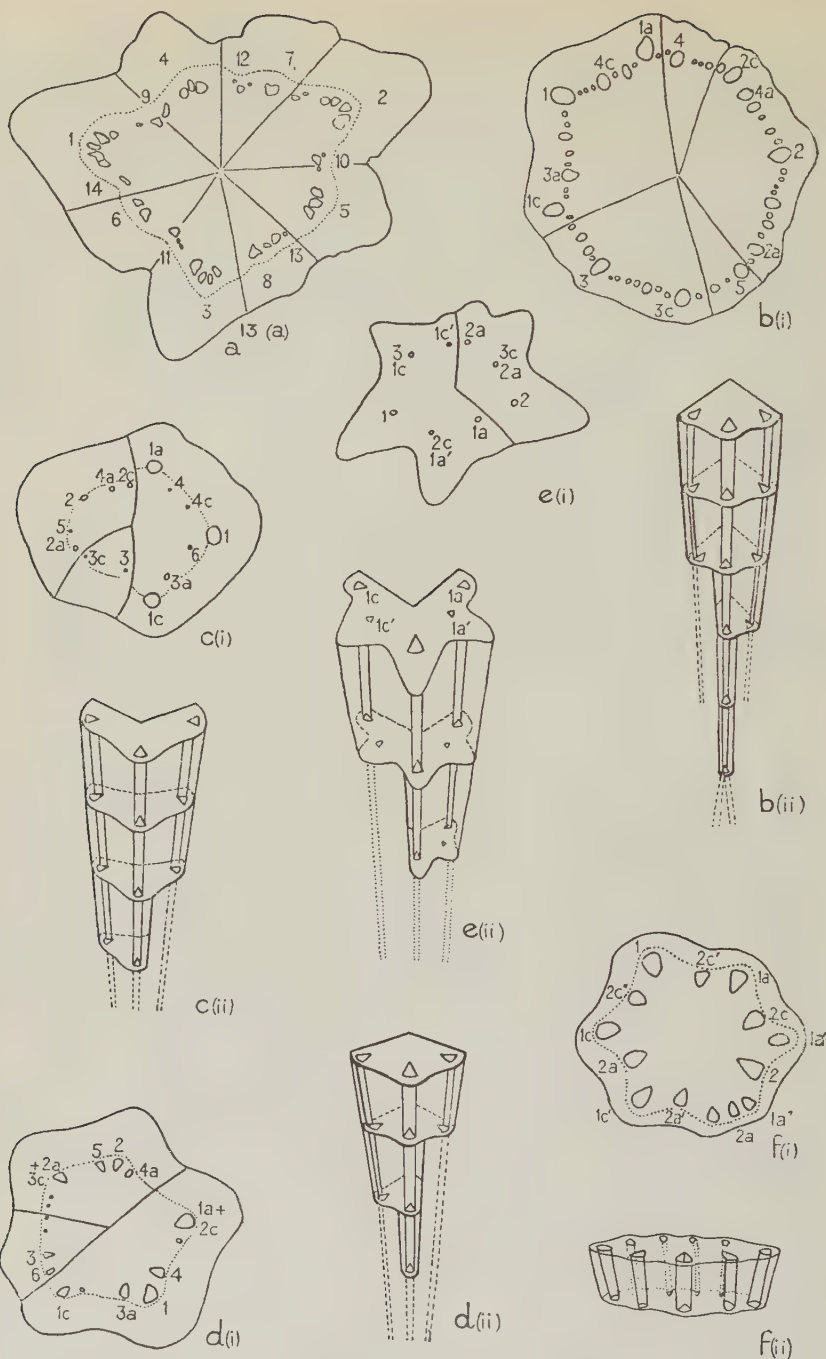
As the differentiation of a new primordium continues, it is synchronized throughout primordium and foliar foundation, and the *tempo* and character of the process seems to be determined by the succession of stages which can be noted in the prodesmogen—desmogen—vascular strands which form the vascular system common to primordium and to the trace system in the axis. Thus, in growth, the shoot system would appear to be constructed of a series of successive, similar, coalescing structures, or growth units, of which the vertical extent in the axis depends upon the transverse and longitudinal extension undergone by that region of the axis spoken of as the foliar foundation. If internodal extension occurs, then the longitudinal extension of this unit may be considerable. Priestley (1929), studying particularly a shoot with  $1/2$  phyllotaxis, regarded this unit as extending in the axis downwards from the leaf insertion at the node to the leaf gap above the next leaf in the same orthostichy. Traced downwards, particularly in more crowded phyllotaxis systems, the area of ground tissue of which the differentiation is influenced by this trace system progressively shrinks and becomes difficult to identify when it no longer includes any of the surface tissues. Thus Griffiths and Malins (1930) were able, by following the progress of internodal extension, to show that, whilst the growth units in decussate types could be identified in some species, as in *Syringa*, through two internodes and thus to the top of the next leaf gap in the same orthostichy, in other types, such as *Mentha*, the course



of the growth unit in the lower internode was completely submerged below the surface. Thus while the growth unit may be regarded theoretically as continuing as far as the next leaf insertion in the same orthostichy, its tangential extension rapidly diminishes downwards and may be lost from the surface of the stem quite rapidly, particularly if each unit, as in *Heracleum*, is undergoing considerable tangential expansion. Thus, so far as its extent is to be judged by the external contour of the stem, it is easily possible to select a series of types amongst plants with the usual spiral systems of phyllotaxis which pass from types with narrow leaf insertions and considerable longitudinal extension, such as *Cheiranthus*, through others with broader leaf insertions to culminate in a type like *Heracleum*, in which the tangential expansion of the unit immediately below prevents any other unit from reaching the surface of the internode.

The figures upon which the following series of comparison are drawn are based partly upon studies made in Leeds, but mainly upon the observations of Nägeli (1858-68). The series illustrate types with an increasing tendency for the primordium to spread round the apex and thus to overlap the marginal regions associated with younger primordia above. Except for *Cheiranthus*, all these types have a trace system of more than one bundle and in the figures the anodic lateral strands are denoted by *a*, *a'*, &c., and the cathodic by *c*, *c'*, &c.

In *Cheiranthus* (Text-fig. 13 *a*) the spiral phyllotaxis approximates to  $5/13$  and each trace consists of a single bundle. Each bundle follows a vertical course until another leaf is inserted below on the same orthostichy, when the bundle from the higher leaf either forks or is diverted to one side of the gap above the incoming trace bundle. In Text-fig. 13 *a* the trace bundles of thirteen leaves may be recognized and it is probable that the bundle of the 14th was diverted to one side of that of leaf 1. The stem is sharply ridged, the three most conspicuous ridges being continuous upwards with the median regions of the three leaves inserted next above this level; in all, eight ridges may be recognized and these overlie the median trace bundles of the eight leaves inserted next above. Each such ridge is evidence that the trace bundle running beneath it affected the primary growth and differentiation of the ground tissues in that sector; in Text-fig. 13 *a* the eight sectors which appear to have differentiated around the eight most conspicuous trace bundles are marked out by radii. The remaining five trace bundles of leaves 9 to 13 above do not run beneath superficial ridges and are evidently running at this level in a part of the axis, the whole of the ground tissues of which underwent primary differentiation in association with the eight trace bundles of the leaves immediately above. If the unit of shoot growth is defined as the leaf and the tissues which differentiate around its trace in the axis, the unit in *Cheiranthus* appears to be the leaf and a sector of the axis which tapers from above downwards and extends vertically through eight internodes; below this point the trace bundle may still be followed, but merely as a bundle running in part of the axis composed of older units.



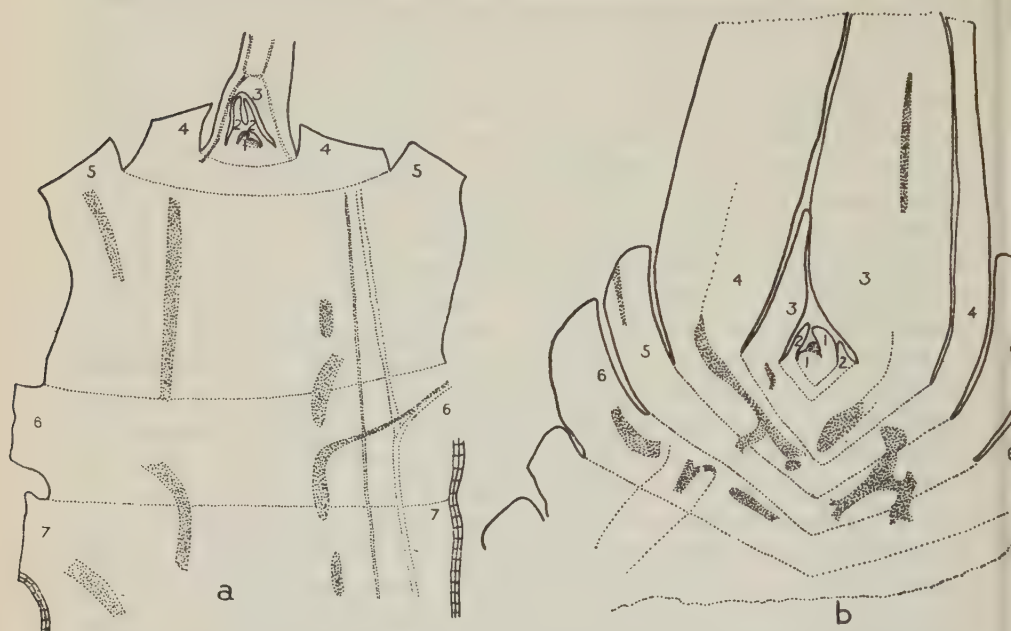
TEXT-FIGS. 13 a-f. Transverse sections of the axis to show the relation of the trace bundles to superficial ridges and reconstructions of the units of shoot growth. 13 a. *Cheiranthus*. 13 b (i) and (ii). *Delphinium*. 13 c (i) and (ii). *Prunus*. 13 d (i) and (ii). *Paullinia*. 13 e (i) and (ii). *Passiflora*. 13 f (i) and (ii). *Heracleum*. (c (i), d (i), and e (i) after Nägeli.)

A young plant of *Delphinium* was found to have  $2/5$  phyllotaxis and a 3-bundle trace. In cross-section (Text-fig. 13 *b*) the trace bundles of a cycle of leaves are indicated, and it will be seen that the median trace bundles are associated to some degree with ridges on the internode, so that five systems are represented at any one level. But unit 1 (the oldest at the level of the section) and unit 2 are associated with three superficial ridges each, the ridges overlying the three bundles of each trace; unit 3 has only two ridges, as its anodic margin has been overlapped by the cathodic of 1 and the anodic bundle of 3 is now running as an extension in tissue already differentiated under the influence of the trace of 1; similarly, unit 4 is overlapped on either side by the anodic margin of 1 and the cathodic margin of 2 respectively. Thus the unit in this case may be depicted as a wide structure through two internodes; it is narrowed sharply at the next node by the overlap of one of its margins, and at the next node by overlap of the other margin (Text-fig. 13 *b*, (ii)). Further stages of this process may be seen by examination of the cross-section and constructed unit in *Prunus* (Text-fig. 13 *c* (i), and *c* (ii)), *Paullinia* (Text-fig. 13 *d* (i) and *d* (ii)), and *Passiflora* (Text-fig. 13 *e* (i) and *e* (ii)). In *Prunus* and *Paullinia* even the median strand of the trace influences primary development to the surface for only three internodes and in *Passiflora* for only two.

In *Heracleum* the ribbing of the surface over the main trace bundles is only seen in the long internodes of the inflorescence axis, but the construction of the short vegetative axis is essentially similar. Text-fig. 13 *f* (i) shows how the trace bundles in the vascular ring lie in a more peripheral position than the remaining bundles which are associated with the higher leaves. Comparison of this type with those already considered shows that all the bundles of the next higher leaf 2 differentiate, after a course of only one 'internode' (a very short course in the vegetative shoot where node and internode are only represented by the zone of leaf insertion), in a region, the whole of which has already undergone its primary differentiation under the influence of the trace of leaf 1. Thus the unit here reaches the greatest possible transverse extent and the least possible vertical course and is a complete disc with a vertical height of only one internode (Text-fig. 13 *f* (ii)).

An encircling leaf insertion is comparatively rare in the dicotyledons; it is the usual type in the monocotyledons; it is therefore very interesting to note that the structure of the axis remains typically dicotyledonous in character in *Heracleum*. This point can be emphasized by contrasting two vegetative axes, both of which have a similar geophilous construction with crowded leaf insertions, e.g. *Heracleum* and *Galanthus*, in median longitudinal section (Text-fig. 14). In both types the increase in girth of the axis is in part due to the expansion of the pith, but there the resemblance ceases; in *Heracleum* it is seen that the bulk of the rest of the axis arises from the activity of that meristematic ring, that, beginning as prodesmogen, later continues its activity as cambium; outside this there is a narrow zone of cortex. In *Galanthus* it is hardly possible to distinguish the limits of cortex and pith, as these grade into

a wide zone of parenchymatous tissue, traversed by leaf-trace strands, and the increased girth of the axis has been the result of long continued cell division in parenchymatous tissue—the primary thickening growth of Helm (1931). Thus the increase in the axis in *Galanthus* is not associated with a vigorous



TEXT-FIG. 14 *a* and *b*. 14 *a*. *Heracleum*. Longitudinal section to illustrate the disc-like units of shoot growth as an extreme dicotyledonous type. 14 *b*. *Galanthus* with funnel-shaped units characteristic of the monocotyledon. ( $\times 30$ .)

growth of the prodesmogen strands. From a very early stage these are seen as isolated strands in actively dividing parenchyma, the increase of these strands in the transverse plane is strictly limited, though their growth easily keeps pace with the longitudinal increase in the tissues in which they lie. This method of growth leaves the course of the leaf-trace strands very clear in the mature axis, no obscurity arising from subsequent prolonged cambial activity, and the course of the leaf-trace strands immediately suggests that the foliar foundations in which they lie enclose one another as a series of funnels, which develop together quite smoothly in the axis, as the parenchyma of one grades insensibly into the similar tissue of the next within or without.

Such a series of funnels, as phytonic units, have been identified by Priestley Scott, and Gillett (1935) in *Alstroemeria* where the phyllotaxis of the erect shoots was  $3/8$ ; in *Galanthus* with a  $1/2$  phyllotaxis the construction is simpler and at any level two funnels are represented in the cross-section. The significant difference in the growth of the monocotyledon and dicotyledon types thus seems to centre around the relative growth activity of the provascular



meristems. In dicotyledons these meristems, first as the prodesmogen ring or cylinder, then as desmogen and subsequently as the cambium, are mainly responsible for the asymmetric growth to form the foliar foundation, which develops further to give rise to a sectorial outgrowth from the flank of the original apex. Even when this excessive prodesmogen growth spreads around so as to involve contributions from the whole cylinder to pass into the same leaf primordium, at all points the new activity separates from the flank of the original cylinder which is maintained in its integrity and subsequently gives rise to the single vascular ring of the dicotyledon axis. (Obviously there are modifications in the developmental plan to include such dicotyledonous types as *Piper*, but the predominance of the single-ring type in the dicotyledons will be generally recognized.)

In monocotyledons these provascular meristems play but little part in the continuing expansion of tissue characteristic of the foliar foundation. This is due instead to a steady increase by division in all planes, but at first especially in longitudinal planes, of relatively iso-diametric, partially vacuolated cells, of exactly the same type in foliar primordium and foundation. Thus a belt or cylinder of tissue is differentiated which completely encloses the axis and within which similar cylinders develop; as they grow upward they expand in periphery, thus forming funnels rather than cylinders, and from the outset the relation of the leaf primordium to the axis is different from that in the dicotyledon. Beginning as it does in more superficial tissue, very commonly in the dermatogen, each new phyton from the outset has the form of a peripheral arc of the apex, rather than a transversely extended sector reaching from periphery to pith. Such arcs arise in succession *within* one another, so that as each arc (often at an early stage a ring) extends in vertical depth, expanding always as it grows forward, it grows into a funnel or a portion of a funnel, enclosed below by older, similar growth units, and itself enclosing in its turn younger ones within. Thus, typically, no single cylinder of vascular strands will traverse the axis of the monocotyledon, but through it will run at varying radial depths, a number of isolated strands which contribute but little to the total thickness of the axis, but by their course, on analysis, yield evidence as to the phytonic construction of the shoot.

This comparison of *Galanthus* and *Heracleum* may certainly be generalized to cover the comparison between umbellifer and monocotyledon. In the typical umbellifer the characteristic encircling leaf arises from the vigorous embellishment of the original prodesmogen ring, which thus early gives rise to a multi-lacunar trace system contributing to an encircling leaf primordium. This leaf system is inserted upon a typical dicotyledon vascular cylinder, composed of trace and synthetic bundles which are early combined into a wide ring of secondary vascular tissue as the result of typical cambial activity. The further characteristics of the vegetative axis, with its well developed phloem and conspicuous food storage, are associated with the geophilous habit.

## SUMMARY

The anatomy and development of *Heracleum* has been intensively studied as a dicotyledon type possessing leaf insertions completely encircling the stem. In the vegetative axis there is no internodal extension and in the bud, as the leaves overarch the axis, the ensheathing bases appear in cross-section as concentric rings which seem to coalesce with the axis (the leaf cushions). As the bud expands to maturity these ensheathing bases are thrust outward, whilst the trace systems diverge horizontally into them, and ultimately these coalescent leaf cushions are separated from the axis by a layer of cork within which is left a typically dicotyledonous ring, composed of trace bundles from the leaf above alternating with synthetic strands associated with higher leaves.

The axillary buds arise on the adaxial surface of the leaf primordium; the bud-trace system ultimately forms a girdle round the axis at the level of the leaf insertion, persisting after the leaf disappears as a characteristic bud cushion.

The internodes are long in the inflorescence axis and have conspicuous collenchymatous ribs.

Terms relating to shoot development are defined, and it is shown that the apex consists of a self-perpetuating group of central initial cells, surrounded by a cylinder of more active flank meristem from which the primordia originate.

In dicotyledons differentiation and growth in the shoot takes place around leaf and trace bundles which thus build up the axis as a succession of growth units (phytons). As the unit widens tangentially in types with wide leaf insertions its vertical extent decreases, and in this sense *Heracleum* is the culmination of such a series in which the unit extends downwards through only one internode. *Heracleum* differs from monocotyledon types in that the axial expansion is due mainly to provascular and then to cambial growth activity, whilst in monocotyledons it is due to continued divisions in the parenchyma cells surrounding the vascular strands.

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## DESCRIPTIONS OF PLATE I

Illustrating the article by G. P. Majumdar on The Organization of the Shoot in *Heracleum* in the Light of Development.

Fig. 1. *Heracleum Sphondylium* L. Rootstock with inflorescence scars bounded by bud cushions.

Fig. 2. *H. Sphondylium*. Longitudinal section of the bud. ( $\times 80$ .)

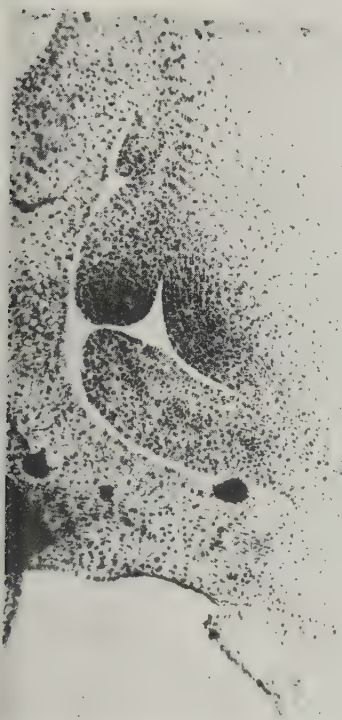
Fig. 3. *H. Sphondylium*. Longitudinal section of the shoot apex to show the region of central initial cells (left), flank meristem (right), and vacuolating file meristem (base). ( $\times 390$ .)



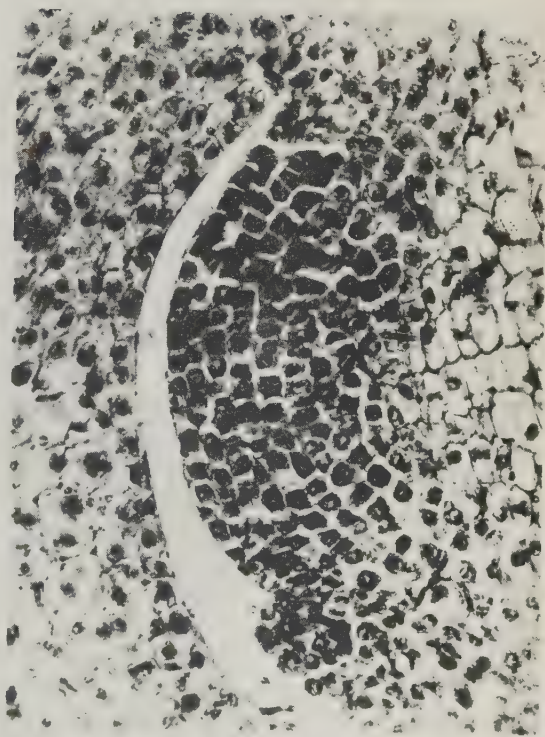




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Huth, Stubbs X. Kent.

MAJUMDAR — HERACLEUM.



# Neglected Generic Characters in the Family Cornaceae

BY

J. HUTCHINSON

With five Figures in the Text

IN 1909 J. Parkin read a comprehensive paper<sup>1</sup> on the inflorescence, in which he pointed out that the study of this feature from the evolutionary point of view had been strangely neglected. The nature of the inflorescence has also not been as fully employed by taxonomists in the delimitation of genera as it might have been. We all know of course that certain families are recognized by a special type of inflorescence, for example, Umbelliferae and Compositae, and to a less extent the Boraginaceae (*sensu stricto*),<sup>2</sup> so that as a character it may be regarded as of considerable importance.

Sometimes even whole tribes of other families are distinguished by their inflorescence, such as the Naucleae in Rubiaceae, in which the flowers are collected into a globose head. In the family Cornaceae, however, similar differences in the inflorescence have not been regarded by some botanists as even of *generic* value. Most species of *Cornus* are of the same general type as the Common Dogwood in Britain, *Cornus sanguinea* Linn., in which the flowers are arranged in corymbose cymes entirely devoid of bracts or bracteoles. Quite three-quarters of the species of the genus, as understood in the systems of Bentham and Hooker and of Engler, have an inflorescence of this type. These comprise the subgenus *Thelycrania* Endl. as delimited by Wangerin in Engler's 'Pflanzenreich'.

The remainder of the species possess inflorescences of a very different, and much more advanced type, i.e. a *capitulum* subtended by an *involucre* of herbaceous or petaloid bracts. As the leaves in all these species are opposite, the bracts number four, six, or eight, as might be expected because of their derivation from and modification and approximation of the upper pairs of foliage leaves due to the suppression of their internodes. This is a great advance on the bractless cyme of *C. sanguinea* and numerous allied species.

More than one modern botanist,<sup>3</sup> however, has ventured to dissent from the generally accepted generic conception of *Cornus*. In an account of the

<sup>1</sup> The full paper was not published until 1914 (Journ. Linn. Soc., xlii. 511).

<sup>2</sup> Excluding *Ehretiaceae*.

<sup>3</sup> Some American botanists have recognized one or more of these small segregate genera.



FIG. 4. Approximate ranges of A, *Cynoxylon*, penultimate climax genus, and B, *Dendrobenthamia*, climax genus.

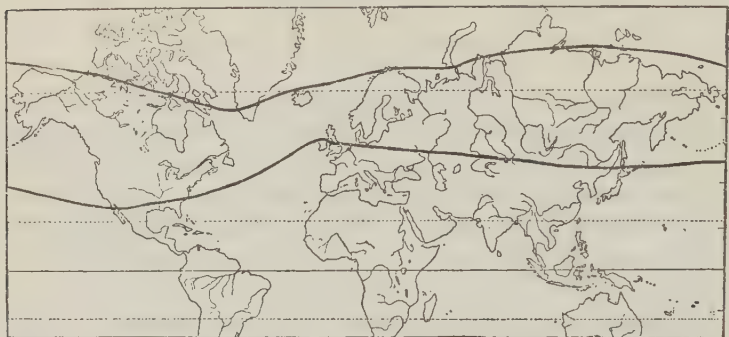


FIG. 3. Approximate range of *Chamaepericlymenum*; in warmer latitudes only at high elevations; stems annual from a rhizome.



FIG. 2. Approximate ranges of A, *Macrocarpium*; note the discontinuous distribution of this least advanced genus; and B, *Afrocrania*, at high elevations in the East African mountains.

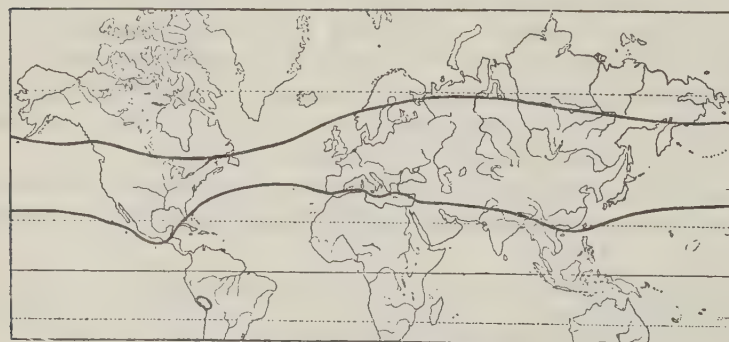


FIG. 1. Approximate range of the genus *Cornus* (*sensu stricto*); two isolated species in Peru and Bolivia.





FIG. 5. Diagrams showing the evolution of the inflorescence from a bractless cyme to a bracteate capitulum in *Cornus* and allied genera: 1. *Cornus* (*sensu stricto*). 2. *Chamaepericlymenum*. 3. *Macrocarpium*. 4. *Afrocrania*. 5. *Cynoxylon*. 6. *Dendrobenthamia*.

Cornaceae in Japan in 1909 T. Nakai<sup>1</sup> remarks: 'I presume that no other phanerogamous plants have, like the so-called genus of *Cornus*, such strikingly different types of species in the same genus. New classifications have often been attempted by many eminent botanists, but methinks the light of the modern systematic botany has hardly applied here. This unfortunate oversight obliges me to disagree with them in many points and express my humble opinions in the following lines.'

Nakai gave a key to the genera represented in Japan, and from *Cornus* he separated *Macrocarpium* Nakai, *Arctocrania* Nakai, and *Benthamia* Lindl. Unfortunately under International Rules neither of the last two names can be maintained, as may be gathered from the enumeration which follows. To these genera recognized by Nakai I have added *Afrocrania*, represented by a solitary species completely isolated in the high mountains of East Tropical Africa where no other Cornaceae are found. And I have split his *Benthamia* into two equally distinct genera, *Cynoxylon* of Rafinesque, and true *Benthamia*, which I have renamed *Dendrobenthamia*, because *Benthamia* had been used twice previously as a generic name, by A. Richard, for an orchid, and by Lindley himself for a Borage.

In view of the separation of these small genera from *Cornus*, it is necessary to reconsider the question of the type species of the genus. As botanical nomenclature dates from 1753 we need go no farther back than the 'Species Plantarum' of Linnaeus. In that work he enumerated the following five species: *C. florida* Linn., *C. Mas* Linn., *C. sanguinea* Linn., *C. suecica* Linn., *C. canadensis* Linn. In the 'Illustrated Flora of the Northern United States and Canada' the late N. L. Britton selected *C. Mas* Linn. as the type species of the genus. When making this arbitrary selection he probably did not consider that the genus might be again subdivided, when it would be found that *C. Mas* belonged to a very small and specialized group and was not typical of the genus as a whole. In fact, *C. Mas* has only three other nearly related species, *C. officinalis* Sieb. and Zucc., *C. chinensis* Wangerin, and *C. sessilis* Torr., belonging to Spach's subgenus *Macrocarpium*. If he had considered the whole genus, therefore, he would no doubt have selected *C. sanguinea* Linn. as the type, because there are thirty-two species with the same general morphology, particularly with respect to the inflorescence and fruit. It seems desirable, therefore, that when a type has to be chosen from a number of species included by the author in the original conception of the genus, a taxonomic study should be made before one is selected. Consequently, it seems better to regard *C. sanguinea* Linn. as the typical species of the genus if the smaller segregate genera are treated as distinct.

After a comprehensive study of the whole family and the nearly related Araliaceae I am in complete agreement with Nakai. In connexion with this view I might mention that lately ('Botanical Magazine' t. 9623; 1941) I have called attention to the fact that in nearly every family there exist one or more

<sup>1</sup> Nakai in Jap. Bot. Mag., xxiii. 35 (1909).

genera which are 'dumping grounds' for species which do not readily fit in with the genera previously established or which are associated by a single character. One example has become quite notorious, the genus *Mesembryanthemum* (Ficoidaceae), in which the late Dr. N. E. Brown brought to light a very great number of important and quite overlooked characters. Other examples are the genera *Kalanchoe* and *Cotyledon*, in the family Crassulaceae, to which every species with a sympetalous corolla has been referred regardless of other characters. And there are many parallel cases familiar to taxonomic botanists who deal with the phanerogamic flora of the whole world or large portions of it, such as tropical Africa and tropical America.

Arising out of this taxonomic study some interesting points regarding the distribution of these small genera have come to light. These seem to be particularly significant because they appear to support the classification based on the morphology and probable phylogeny.

*Cornus*, in a restricted sense (i.e. of the *C. sanguinea* type) is widely distributed in the temperate regions of the northern hemisphere, extending southwards on the American Continent as far as Guatemala, in Europe south to Sicily and northern Greece, and in Asia to Syria and northern Persia, the Himalayas, and central China. Comparatively recently two outlying species have been described from South America, one in Bolivia, the other in Peru<sup>1</sup> (see Fig. 1).

The first and most primitive segregate genus, *Macrocarpium*, has a very interesting discontinuous distribution, namely, central and south Europe and Asia Minor, western China, Japan, Korea, and California. Not only from the structure of the inflorescence, therefore, but from the distribution we may infer that this is a relatively old genus of which only the remnants are left (see Fig. 2).

The distribution of the isolated monotypic tropical African genus *Afrocrania* is also shown on this map. *Afrocrania* is remarkable not only on account of its dioecious flowers, but by the fact that the male and female inflorescences are different, the male being composed of small cymules, whilst the female is umbellate.

The map of Fig. 3 shows the approximate range of the herbaceous genus *Chamaepericlymenum*, which is admirably adapted to the cold climates in which it is found. In this the inflorescence is still composed of small cymules or umbels, but the bracts are advanced in that they are petaloid, a compensating character to make up for the loss of size in the inflorescence and flowers, and the fruits are baccate and attractive.

Then the two more highly evolved genera are found in widely separated areas and may thus have arisen independently from the parent stock. These are the climax genera *Cynoxylon* (see A of Fig. 4) and *Dendrobenthamia* (see B of Fig. 4).

<sup>1</sup> This was collected by Matthews as long ago as before the middle of last century, but has lain unnamed in the Kew Herbarium ever since.



The following key shows the differences among these genera:

Inflorescence a corymbose cyme, without bracts . . .	CORNUS.
Inflorescence capitate, with 4 or more herbaceous or petaloid bracts before or during flowering:	
Flowers pedicellate, in umbelliform cymules or umbels:	
Trees or large shrubs, with precocious hermaphrodite flowers, arranged in umbels within the herbaceous involucre bracts . . . . .	MACROCARPIUM.
Trees; flowers dioecious, the males within the herbaceous early deciduous bracts in short cymules, the females fewer and umbellate . . . . .	AFROCRANIA.
Herbs with annual stems from a perennial rhizome; bracts petaloid; flowers in small cymules or umbellate . . . . .	CHAMAEPERICLYMENUM.
Flowers sessile, within the usually petaloid bracts:	
Calyces and fruits free from one another . . .	CYNOXYLON.
Calyces and fruits united into a fleshy syncarp. . .	DENDROBENTHAMIA.

**Macrocarpium** Nakai in Bot. Mag. Tokyo, xxiii. 38 (1909). *Cornus* subgen. *Macrocarpium* Spach Hist. Veg. Phan. viii. 101 (1839); Koehne Dendrol. 435 (1893); Harms in Engl. and Prantl. Pflanzenfam. 3, viii. 266 (1898). *Cornus* sect. *Tanycrania* Endl. Gen. 798 (1839), et Ench. 397 (1841), partim.

Arbores vel frutices, pilis adpressis bifurcatis pubescentes; folia decidua, opposita, integra, penninervia; flores praecoces, hermaphroditi, flavi, umbellati, bracteis herbaceis vel coriaceis post anthesin mox deciduis involucreti; calycis dentes minimi; petala 4, parva, valvata; stamina 4, filamentis subulatis, antheris oblongis; discus pulvinatus; ovarium 2-loculare; stylus breviter columnaris, stigmatibus capitato; ovula in loculis solitaria; drupae oblongae, inter se liberae, putamine osseo; semina oblonga.

Species 3, central and southern Europe, Asia Minor, western China, Japan and Korea, California.

Type species *M. Mas* (Linn.) Nakai.

**Macrocarpium mas** (Linn.) Nakai in Bot. Mag. Tokyo, xxiii (1909). *Cornus mas* Linn. Sp. Pl. 117 (1753). *C. mascula* Zorn. Ic. Pl. Med. t. 129 (1779). *C. vernalis* Salisb. Prodr. 66 (1796). *C. praecox* Stokes Bot. Mat. Med. i. 222 (1812). *C. flava* Steud. Nomencl. 227 (1821). *C. nudiflora* Dumort. Fl. Belg. 83 (1827). *C. erythrocarpa* St. Lag. in Bull. Soc. Bot. Fr. xxxi, Bibl. 201 (1883). *C. homericia* Bub. Fl. Pyren. ii. 337 (1900).

For further references and pre-Linnean literature see Wangerin in Engl. Pflanzenr. l.c. (supra).

**Distribution.** Central and south Europe and Asia Minor (France, central Germany, Luxembourg, Belgium, Switzerland, Austria, Bohemia, Hungary,



Galicia, south Russia, Italy, south-east through northern Greece to the Caucasus and Armenia.

This species is known on the Continent as 'Kornelkirsche', 'Herlitzte', 'Knorpelkirsche', and 'Cornouiller mâle', and flowers in March and April. It has been cultivated in Britain for many centuries and is called the 'Cornelian Cherry'. There are several garden varieties, including a dwarf form and some with variegated leaves. In favourable seasons it flowers as early as February. Bean (Trees and Shrubs, ed. 2, 391) says that, because of its leafless state at the time of flowering, it should be associated with evergreens. The fruit has been used to make a preserve.

There is a coloured picture of this species in Curtis, Bot. Mag. t. 2675.

**Macrocarpium officinale** (Sieb. et Zucc.) Nakai in Bot. Mag. Tokyo, xxiii. 38 (1909). *Cornus officinalis* Sieb. et Zucc. Fl. Jap. i. 100, t. 50 (1835); Wangerin in Engl. Pflanzenr. 4, ccxxix. 80 (1910).

*Distribution.* Japan and Korea.

This is known in Japan as 'Sandzaki', where it flowers in April. Bean (l.c.) says it is scarcely distinguishable when in flower from *M. mas*. It is interesting that two so closely allied species are so far separated geographically, probably due to extinction during the glacial periods.

**Macrocarpium chinense** (Wangerin) Hutch. comb. nov. *Cornus chinensis* Wangerin in Fedde Repert. Nov. Sp. vi. 100 (1908), et in Engl. Pflanzenr. 4, ccxxix. 80 (1910).

*Distribution.* Western China (Szechuan and E. Tibet to Hupeh).

**Macrocarpium sessile** (Torr. ex Dur.) Nakai, l.c. *Cornus sessilis* Torr. ex Dur. in Journ. Acad. Nat. Sci. Philadelph. 2, iii. 89 (1855), et in Pac. R. Rep. iv. 94, t. 7 (1856); Wangerin in Engl. Pflanzenr. 4, ccxxix. 81 (1910).

*Distribution.* Pacific United States: North California.

This was introduced into cultivation in this country in 1903, but is not so well known as *M. mas* and *M. officinale*. It flowers in its native place from March to April.

**Afrocrania** Hutch. gen. nov.

*Cornus* subgen. *Afrocrania* Harms in Engl. et Prantl Pflanzenfam. 3, viii. 266 (1898); Wangerin in Engl. Pflanzenr. 4, ccxxix. 76, fig. 19 (1910).

Arbor; folia opposita, petiolata, ovato-lanceolata, integra, penninervia, pubescentia; flores dioici, masculi in cymulis terminalibus subumbellatis, feminei in umbellis dispositi, bracteis 4 ovatis albidis subherbaceis involu-crati; calycis dentes triangulares; petala valvata, ovata; stamina 4, erecta; filamenta subulata; antherae ellipsoideae; discus conspicuus, pulviniformis, styli rudimento subulato coronatus; ovarium 2-loculare, adpresse sericeum; ovula solitaria, pendula; stylus crassus, cylindricus, stigmatibus breviter bilobato;

drupae umbellatae, pedicellatae, ovoideo-oblongae, calyce et stylo coronatae, putamine crustaceo 2-loculari.

Species 1, mountains of east Tropical Africa.

**Afrocrania Volkensii** (Harms) Hutch. comb. nov. *Cornus Volkensii* Harms in Engl. Pflanzenfam. 3, viii. 266 (1898); Wangerin in Engl. Bot. Jahrb. xxxviii. Beibl. No. 86: 12-13, et 52 (1906), et in Engl. Pflanzenr. 4, ccxxix. 76, fig. 19 (1910).

*Distribution.* Kenya Colony: Mt. Kenya (2,300-2,600 m.); Mt. Elgon (2,600-3,200 m.); Mt. Aberdare (3,300 m.); Uganda: Ruwenzori (2,700-3,600 m.). Belgian Congo: Virunga Mts. (2,600-2,900 m.): Tanganyika Territory: Uluguru Mts. (1,600-2,200 m.); Mt. Rungwe (2,100-2,500 m.). Nyasaland: Mt. Milanji.

**Chamaepericlymenum** Graebn. in Aschers. et Graebn. Fl. Nordost-deutsch. Flachl. 225 et 539 (1898). *Cornus* sect. *Arctocrania* Endl. Gen. 798 (1839); Wangerin in Engl. Pflanzenr. 4, ccxxix. 81 (1910). *Cornus* sect. *Cornion* Spach Hist. Veg. Phan. viii. 103 (1839). *Cornella* Rydb. in Bull. Torr. Club, xxxiii. 147 (1906). *Arctocrania* Nakai in Jap. Bot. Mag. Tokyo, xxiii. 39 (1909).

Herbae perennes, humiles; folia opposita vel verticillata; flores hermaproditii, in cymulis umbelliformibus dispositi, bracteis 4 amplis albis involu-crati; calyx 4-dentatus; petala 4, valvata; stamina 4; antherae oblongae; discus pulvinatus; ovarium 2-loculare; stylus columnaris, stigmatibus capitato; ovula in loculis solitaria, pendula; fructus ovoideus vel oblongus, mesocarpio exsucco, putamine osseo vel crustaceo.

Species 2, circumpolar in the northern hemisphere. Type species *C. suecicum* (Linn.) Aschers. et Graebn.

This is the only herbaceous genus of Cornaceae, and is a striking example of the evolution of herbs from woody ancestors, as no doubt it is. Parallel types are not wanting in woody genera of other families, such as Tetracera (Dilleniaceae), Potentilla (Rosaceae), Ochna (Ochnaceae), &c.

**Chamaepericlymenum suecicum** (Linn.) Aschers. et Graebn. l.c. 539 (1898); Britton and Brown Fl. Northern States and Canada, ii. 665, with fig. (1913). *Cornus suecica* Linn. Sp. Pl. 118 (1753); Wangerin l.c. (supra); Druce Comital Fl. Brit. Isl. 146 (1932). *C. borealis* Gorter Fl. Ingr. 24 (1761). *C. herbacea* Oeder. Fl. Dan. t. 5 (1766). *C. biramis* Stokes Bot. Mat. Med. i. 221 (1812). *Cornella suecica* Rydb. in Bull. Torr. Club, xxxiii. 147 (1908). *Arctocrania suecica* Nakai, l.c. (1909). For further references and pre-Linnean names see Wangerin, l.c.

*Distribution.* From Labrador and Newfoundland, through Greenland, Iceland, north Britain, across northern Europe and northern Asia to north Japan, Kamtschatka, and Alaska; in Britain only as far south as north Lancashire and north-east Yorkshire.

It is rather unfortunate that a British plant should have to be known by such a long generic name, resuscitated from the obscurity of pre-Linnean botany by Graebner in 1898. Otherwise Rydberg's name *Cornella* could have been used, though the old sectional name *Arctocrania*, employed by Nakai, was very appropriate considering the circumpolar distribution.

**Chamaepericlymenum canadense** (Linn.) Aschers. et Graebn. Fl. Nordostdeutsch. Flachl. 799 (1898); Britton and Brown Fl. Northern States and Canada, ii. 664, with fig. (1913). *C. unalaschkense* (Ledeb.) Rydb. Fl. Rocky Mts. and Adjac. Plains, 635 (1917). *Cornus canadensis* Linn. Sp. Pl. 118 (1753); Wangerin in Engl. Pflanzenr. 4, ccxxix. 83 (1901). *C. unalaschkensis* Ledeb. Fl. Ross. ii. 378 (1844-6). *C. herbacea canadensis* Pall. Fl. Ross. i. 52 (1784). *Cornella canadensis* Rydb. Bull. Torr. Club, xxxiii. 147 (1906). *Arctocrania canadensis* (Linn.) Nakai in Jap. Bot. Mag. xxiii. 40 (1909). For further references and pre-Linnean synonymy see Wangerin, l.c.

*Distribution.* From Manchuria, Sakhalin, and Japan, through Alaska and Canada to Labrador and Newfoundland; in the United States on the mountains south to Virginia, north California, and Colorado.

**Cynoxylon** Raf. Alsogr. Amer. 59 (1838); Britton, North American Trees, 744 (1908). *Benthamia* Spach Hist. Veg. Phan. viii. 106 (1839). *Cornus* sect. *Tanycrania* Endl. Gen. 798 (1839), et Ench. 397 (1841), partim. *Cornus* subgen. *Benthamia* Wangerin in Engl. Pflanzenr. l.c. 86 (1910). *Cornus* subgen. *Discocrania* Harms in Engl. and Prantl. Pflanzenfam. 3, viii. 267 (1898); Wangerin, l.c. 84.

Arbores vel frutices; folia opposita; flores hermaphroditi, capitati, sessiles, bracteis 4-8 magnis albis vel rubescentibus involucrat; calyces minimi, inter se liberi; petala 4, valvata; stamina 4; antherae oblongae; discus pulvinatus; ovaria inter se libera, 2-locularia; stylus columnaris, stigmatibus capitato; ovula in loculis solitaria, pendula; drupae liberae, 2-spermae.

Species 4, North America.

Type species *C. floridum* (Linn.) Raf.

**Cynoxylon disciflorum** (Moc. et Sessé ex DC.) Hutch. comb. nov. *Cornus disciflora* Moc. et Sessé ex DC. Prodr. iv. 273 (1830); Wangerin, l.c. 84, fig. 20. *C. grandis* Cham. et Schlechtd. in Linnaea, v. 171 (1830). *Benthamia disciflora* (Moq. et Sessé) Nakai in Jap. Bot. Mag. xxiii. 41 (1909); *B. grandis* (Cham. et Schlechtd.) Nakai, l.c.

*Distribution.* Mountains of central Mexico and Costa Rica.

In this species the bracts are leathery and fall off at a very early stage leaving behind a circular disc-like plate.

**Cynoxylon floccosum** (Wangerin) Hutch. comb. nov. *Cornus floccosa* Wangerin in Fedde Repert. Nov. Sp. vi. 101 (1908). *C. disciflora* Rose in Contrib. U. St. Nat. Herb. viii. 53 (1903-5), non Moc. et Sessé.

*Distribution.* Mexico.



**Cynoxylon floridum** (Linn.) Raf. Alsog. Amer. 59 (1838); Britton, North Amer. Trees, 744, fig. 682-3 (1908); Britton and Brown, Fl. Northern States and Canada, ii. 664, with fig. (1913); Small, Fl. SE. Un. St. 854 (1913). *Cornus florida* Linn. Sp. Pl. 117 (1753); Wangerin, l.c. 86 (1910). *C. candidissima* Mill. Gard. Dict. ed. 8: n. 6 (1759). *Benthamidia florida* Spach Hist. Veg. Phan. viii. 107 (1839). *Benthamia florida* (Linn.) Nakai in Jap. Bot. Mag. xxiii. 41 (1909). For further references and pre-Linnean names see Wangerin, l.c.

*Distribution.* According to Britton (l.c. *supra*) this is probably the most showy plant of eastern North America. It occurs in forests from Massachusetts and Ontario to Minnesota, south to Florida and Texas; and there is a variety var. *urbiniiana* (Rose) Wangerin (*Cornus urbiniana* Rose, Contr. U.S. Nat. Herb. viii. 53 (1903)) in the temperate regions of southern Mexico (near Orizaba and Vera Cruz).

The wood is hard, tough, and strong, and is a favourite with turners in the manufacture of parts of machinery, wagon-wheel hubs, &c. The bark has been used as a remedy in fevers.

The autumn colour of the leaves is attractive and the scarlet berries much eaten by birds (Britton, l.c.)

**Cynoxylon Nuttallii** (Audubon) Shafer in Britton and Shafer, North Amer. Trees, 746, fig. 684 (1908). *Cornus Nuttallii* Audubon, Birds Amer. t. 467 (1837); Wangerin in Engl. Pflanzenr. 4, ccxxix. 87 (1910), which see for further references. *Benthamia Nuttallii* (Audubon) Nakai in Jap. Bot. Mag. xxiii. 41 (1909).

*Distribution.* A magnificent tree in evergreen forests from British Columbia southward to the mountains of southern California, reaching its greatest development, about 30 metres high, with a trunk diameter of 6 dm. in the northern part of its range (Britton and Shafer, l.c.).

Like *C. floridum*, its wood is hard, close-grained, light red-brown, and satiny, and is used for cabinet work and tool handles.

**Dendrobenthamia** Hutch. nom. nov.

*Benthamia* Lindl. Bot. Reg. t. 1579 (1833), et Veg. Kingd. 783 (1847), nec Lindl. (1830) nec A. Rich. (1828); Nakai in Jap. Bot. Mag. xxiii. 40 (1909), partim. *Cornus* subgen. *Benthamia* Wangerin in Engl. Pflanzenr. 4, ccxxix. 88 (1910).

Arbores parvae; folia opposita; flores hermaphroditi, capitati, sessiles, bracteis 4 magnis petaloideis involucrat; calyces in massam connati; petala 4, valvata; stamina 4; antherae oblongae; discus pulvinatus; ovaria 2-locularia, in syncarpium globosum connata; stylus columnaris, stigmatibus capitato; ovula in loculis solitaria, pendula; drupae 2-spermae, in syncarpium carnosum areolato-tuberculatum confluentes, putaminibus magnis osseis.

Species 3, India to Japan.

Type species *D. capitata* (Wall.) Hutch.



**Dendrobenthamia japonica** (Sieb. and Zucc.) Hutch. comb. nov. *Benthamia japonica* Sieb. and Zucc. Fl. Jap. i. 38, t. 16 (1835), non *Cornus japonica* Thunb. *B. Kousa* (Buerger) Nakai l.c. *Cornus Kousa* Buerger ex Miq. in Ann. Mus. Bot. Lugd. Bat. ii. 159 (1865); Wangerin in Engl. Pflanzenr. 4, ccxxix (1910), which see for further references; *C. japonica* Koehne Dendrol. 438 (1893), non Thunb.

*Distribution.* Japan, Korea, central and western China.

**Dendrobenthamia hongkongensis** (Hemsl.) Hutch. comb. nov. *Benthamia japonica* var. *sinensis* Benth. in Hook Kew Journ. Bot. iv. 165 (1852); *B. japonica* Benth. Fl. Hongkong 138 (1861), non Sieb. and Zucc. *B. hongkongensis* (Hemsl.) Nakai l.c. 41 (1909). *Cornus hongkongensis* Hemsl. in Journ. Linn. Soc. xxiii. 345 (1888); Wangerin, l.c. 89.

*Distribution.* Hongkong.

**Dendrobenthamia capitata** (Wall.) Hutch. comb. nov. *Cornus capitata* Wall. ex Roxb. Fl. Ind. ed. Carey et Wall, i. 434 (1820), et Pl. As. Rar. t. 214 (1832); Wangerin, l.c. 89. *Benthamia fragifera* Lindl. Bot. Reg. t. 1579 (1833). *B. capitata* (Wall.) Nakai, l.c. 41 (1910); Bot. Mag. t. 4641.

*Distribution.* Himalayas, from Kumaon to Assam, Yunnan, and Hupeh.

#### SUMMARY

Attention is called to the importance of the nature of the inflorescence as a generic character, particularly in the genus *Cornus*. The greater number of the species of this genus have a loose corymbose cyme without bracts or bracteoles, and it is proposed that this genus should contain only these, and several others are recognized based on characters derived from the inflorescence. A key to these is provided and maps showing the range of each genus.



## Macrozanonia Cogn. and Alsomitra Roem.

BY

J. HUTCHINSON

THE donation to the Kew museums of some of the beautiful winged seeds of *Macrozanonia macrocarpa* (Blume) Cogn. from New Britain induced me to look into the nomenclature of this and the genus *Alsomitra* Roem. in which it was formerly included. Some notes on this interesting plant were published by the late R. A. Rolfe in the 'Kew Bulletin', 1920, p. 197. It will be clear from what follows that the name *Macrozanonia* cannot be maintained and that certain other species hitherto referred to *Alsomitra* require a new generic name, for which *Neoalsomitra* is proposed.

The taxonomic history may be briefly stated as follows. The species, which in recent works has been known as *Macrozanonia macrocarpa* (Blume) Cogn., was first described by Blume<sup>1</sup> as *Zanonia macrocarpa*. Under *Zanonia* he created for it a new section, *Alsomitra* Blume, distinguished from true *Zanonia* (i.e. *Z. indica* L.) by its large hemispherical fruits with many beautifully winged seeds in each loculus. In true *Zanonia* the fruits are small and cylindrical and have only two seeds in each loculus.

Roemer,<sup>2</sup> in 1846, raised Blume's section *Alsomitra* to generic rank, and he enumerated *A. macrocarpa* as the first species, adding five others, *A. angulata* (*Zanonia angulata* Wall.), *A. timorana* (*Zanonia timorana* Spanoghe), *A. sarcophylla* (*Zanonia sarcophylla* Wall.), *A. clavigera* (*Zanonia clavigera* Wall.), and *A. laxa* (*Zanonia laxa* Wall.).

In 1881 Cogniaux<sup>3</sup> founded a new section of *Zanonia* for *Z. macrocarpa*, which he called *Macrozanonia*, ignoring or overlooking the fact that Blume had already established for it the sectional name *Alsomitra*. And subsequently Cogniaux<sup>4</sup> described this as a new genus *Macrozanonia*. The same treatment has been followed by Cogniaux in Engler, 'Das Pflanzenreich, Cucurbitaceae', published in 1916, and he added another species *M. Clarkei*, which is still imperfectly known. *Macrozanonia* is therefore a synonym of *Alsomitra* Roemer (*Zanonia* section *Alsomitra* Blume), because it is the type species of that genus, and it cannot be maintained even as a sectional name under *Zanonia*, the valid sectional name being *Alsomitra* Blume.

The name *Alsomitra* Roem., therefore, should be restored for *Macrozanonia macrocarpa* (Blume) Cogn., and *M. Clarkei* (King) Cogn., and the new

<sup>1</sup> Blume, Bidjr. 937 (1825).

<sup>3</sup> Cogniaux in DC. Monogr. iii. 927 (1881).

<sup>2</sup> Roemer, Synops. ii. 117 (1846).

<sup>4</sup> Cogniaux in Bull. Herb. Boiss. i. 612 (1893).

name *Neoalsomitra* Hutch. is proposed for the remainder of the species included by Roemer and subsequent authors under *Alsomitra*. These are dealt with in the following.

**Alsomitra** Roemer, Synops. ii. 117 partim (1846). *Zanonia* sect. *Alsomitra* Blume, Bidjr. 937 (1825). *Zanonia* sect. *Macrozanonia* Cogn. in DC. Monogr. iii. 927. *Macrozanonia* Cogn. in Bull. Herb. Boiss. i. 612 (1893), et in Engl. Pflanzenr. 4, cclxxv. 1: 262 (1916).

*Descript. emend. et ampl.* Frutices alte scandentes; ramuli graciles, molliter lignosi. Folia alterna, petiolata, simplicia, late ovata vel ovato-rotundata, basi rotundata vel leviter cordata et trinervia, integra vel trilobata; cirrhi bifurcati, spiraliter torti; petioli basi callo annulari. Flores dioici, masculi paniculati; receptaculum cupuliforme; calyx demum trilobatus; petala 5, valvata, carnosa; stamina 3, libera, disco inserta; filamenta brevissima; antherae erectae, una unilocularis, ceterae biloculares, loculis rectis, connectivo haud producto; pistillodia 3, minuta. Flores feminei racemosi; calyx alabastro clausus, demum in lobos 2-3 irregulariter ruptus et caducus; petala ut in mare; ovarium triloculare; ovula numerosissima; styli 3, bilobati. Fructus magnus, globosus vel ovoideo-cylindricus. Semina pro loculo pluria, magna, compressa, fere orbicularia vel elliptica, ala ampla divaricata membranacea pulchre sericea et nitida cincta.

Species 2, Indo-Malaya.

**Alsomitra macrocarpa** (Blume) Roem. Synops. ii. 117 (1846). *Zanonia macrocarpa* Blume, Bidjr. 937 (1825); Ser. in DC. Prodr. iii. 299 (1828); Miq. Fl. Ind. Bat. i, pt. 1: 683 (1855); Cogn. in DC. Monogr. iii. 927 (1881); Warb. in Engl. Bot. Jahrb. xiii. 444 (1891); Gibbs, Dutch NW. New Guinea, 17, 51, 222 (1917). *Macrozanonia macrocarpa* (Blume) Cogn. in Bull. Herb. Boiss. i. 612 (1893); K. Schum. and Lauterb. Fl. Deutsch. Südsee, 589 (1901); Cogn. in Bull. Soc. Bot. Belg. xliii. 358 (1906), et Engl. Pflanzenr. 4, cclxxv. 1: 262, fig. 63; Rolfe in Kew Bull. 1920: 197. *Zanonia philippinensis* Merr. in Philipp. Journ. Sci. i, Suppl. 241 (1906); Cogn. in Engl. l.c. 264.

Thailand: *Kerr*. Borneo: *Korthals*; *Beccari*. Java: *Blume*; *Zollinger*; *Treub*; *Busse*; *Teijsmann* and *Bennendijk*; and cult. at *Buitenzorg*. Batjan Isl.: *De Vriese*. Sumatra: *Lorzing* 5171. Aru Islands: *Longman*; *Colclough*. New Guinea: *Barton*; *Schlechter* 18317; *Sherring*. Philippines: *Elmer* 9645; 11107; *Clemens* 324; *McGregor* (Bur. of Sci.) 18711; *Mabesa* (For. Bur.) 24997.

**Alsomitra Clarkei** (King), comb. nov. *Zanonia Clarkei* King in Journ. Asiat. Soc. Beng. lxvii. 41 (1898); Mat. Fl. Mal. Penins. 385 (1902). *Macrozanonia Clarkei* Cogn. in Engl. Pflanzenr. 4, cclxxv. 1: 264.

Malay Peninsula: Perak, 90-160 m., fr. January, *Kings Collector* 1230.

Only a fruiting specimen with leaves of this species is so far known; the body of the seed is coarsely pectinately lobed and the wing is probably more



or less orbicular in outline, and not with two spreading lobes as in *A. macrocarpa*.

**Neoalsomitra** Hutch. nom. nov.—*Alsomitra* Roemer Synops. ii. 117, partim (1846), non *Zanonia* sect. *Alsomitra* Blume; Benth. et Hook. f. Gen. Pl. i. 840 (1867); Cogn. in DC. Monogr. Phanerog. iii. 928 (1881); Baill. Hist. Pl. viii. 424 (1886); Pax in Engl. et Prantl, Pflanzenfam. 4, v. 12 (1889); Cogn. in Engl. Pflanzenr. 4, cclxxv. 1: 11 (1916).

Frutices scandentes; folia simplicia vel saepe 3-5-foliolata, foliolis basi interdum biglandulosis; cirrhi simplices vel bilobati; flores dioici, in paniculas vel racemos laxos axillares dispositi. Flores masculi receptaculo cupulari; calyx 5-partitus; corolla rotata, 5-partita, segmentis erosis; stamina 5, libera; filamenta brevissima; antherae oblongae, uniloculares, demum recurvae. Flores feminei; ovarium uniloculare vel imperfecte 3-loculare; styli 3-4, stigmatibus semilunaribus; ovula in placentis numerosa, pendula. Fructus clavatus vel cylindricus, teres vel subtrigonus, apice late truncatus et trivalvis; semina imbricata, compressa, ala tenuissima elongata terminata, marginibus sinuato-tuberculatis, testa crustacea.

Species 22, India to Polynesia and Australia; type species (selected), *N. sarcophylla* (Roem.) Hutch., Indo-Malaya.

The following key has been made from the herbarium material at Kew, some of it very imperfect, and from Cogniaux's key in Engler's Pflanzenreich. Its shortcomings will be obvious.

# Leaves simple:

## Leaves not lobed:

Leaves acuminate, cordate at the base; inflorescence a panicle:

Leaves entire, deeply cordate; Thailand sp. . . . . 1. *simplex*

Leaves denticulate; Timor Isl. sp. . . . . 2. *timorana*

Leaves not acuminate, at most acute:

Leaves truncate or scarcely emarginate at the base, thinly membranous; inflorescence a raceme . . . . . 3. *Schultzei*

Leaves shortly auriculate-cordate at the base, chartaceous . . . . . 4. *simplicifolia*

## Leaves distinctly 3-5-lobed:

Fruits setulose-muricate . . . . . 5. *capricornica*

Fruits smooth . . . . . 10. *plena*

# Leaves 3-5-foliolate:

## Lower leaves 5-foliolate:

Extreme lateral leaflets separately petiolulate . . . . . 6. *angustipetala*

Extreme lateral leaflets on the same petiolules as the 2nd and 3rd lateral leaflets:

Fruits densely puberulous . . . . . 7. *pubigera*

Fruits glabrous:

Leaflets quite separate at the base with separate petiolules:

Fruits 5-6 cm. long; leaflets of inflorescence 3 . . . . . 8. *integrifoliola*

Fruits 7-5 cm. long; leaflets of inflorescence 5 . . . . . 9. *tonkinensis*

- Leaflets pedately connected at the base; fruits 3-3.5 cm.  
 long . . . . . 10. *plena*
- Lower leaves 3-foliate, sometimes the lateral leaflets with a  
 small or very small lobule at the base:
- Leaflets suborbicular, rounded at the apex or minutely  
 apiculate . . . . . 11. *rotundifoliola*
- Leaflets not suborbicular:
- Leaflets fleshy:
- Leaflets ovate, petals acute . . . . . 12. *sarcophylla*
- Leaflets oblong; petals rounded and hairy at the apex . . . 13. *philippinensis*
- Leaflets not fleshy:
- Leaflets not glandular at the base:
- Leaflets subequal in length, the lateral subsymmetrical:
- Branches glabrous; tendrils rather deeply 2-lobed; sepals  
 acuminate . . . . . 14. *Muelleri*
- Branches pubescent; tendrils undivided or only bifid at  
 the apex; sepals acute . . . . . 15. *trifoliolata*
- Leaflets unequal-sized, the lateral shorter and more or less  
 asymmetrical:
- Petioles glabrous:
- Tonkin species (not seen) . . . . . 16. *Balansae*
- Australian species . . . . . 17. *suberosa*
- New Guinea species (not seen) . . . . . 18. *Beccariana*
- Petioles puberulous; Philippines species . . . . . 19. *pubescens*
- Petioles villous; Celebes and New Guinea species . . . . . 20. *Schefferiana*
- Leaflets more or less biglandular at the base . . . . . 21. *clavigera*
- Leaflets and petiolules with several glands . . . . . 22. *Stephensiana*

1. *N. simplex* (Craib), comb. nov.—*Alsomitra simplex* Craib in Kew Bull. 1930: 409.

Thailand: Betong, climbing on trees by stream in evergreen forest, Aug., Kerr 7524.

Until fruits and seeds of this are collected the generic position will remain somewhat uncertain; the leaves are very thin in texture.

2. *N. timorana* (Spanoghe), comb. nov. *Zanonia timorana* Spanoghe in Linnaea, xv. 205 (1841); Miq. Fl. Ind. Bat. 1, i. 683 (1855). *Z. timorensis* Walp. Rep. ii. 194 (1843). *Alsomitra timorana* (Spanoghe) Roem. Syn. Fam. ii. 117 (1846); Cogn. in DC. Mongr. Phan. iii. 935 (1881), et in Engl. Pflanz. 4, cclxxv. 1: 13 (1916).

Timor: *Spanoghe*—Not seen.

3. *N. Schultzei* (Cogn.), comb. nov.—*Alsomitra Schultzei* Cogn. in Engl. Pflanzenr. 4, cclxxv. 1: 12 (1916).

New Guinea: Augusta River, *L. Schultze* 170. Not seen.

4. *N. simplicifolia* (Merrill), comb. nov.—*Alsomitra simplicifolia* Merrill

in Philipp. Journ. Sci. xx. 470 (1922). Philippines: Mindanao, *Ramos & Edano* Bur. Sci. No. 37387. Local name 'lalapid'.

I have only seen a leaf-specimen and one detached fruit. The shortly auriculate-cordate base of the leaves is characteristic. The petiole is inserted at the base on an annular callus as in *Alsomitra macrocarpa* (Blume) Roem.

5. *N. capricornica* (F. Muell.), comb. nov.—*Alsomitra capricornica* F. Muell. Fragm. Phyt. Austral. vii. 61 (1870); Cogn. in Engl. Pflanzenr. 4, cclxxv. 1: 12 (1916).

East Australia: Cleveland, *Cunningham* 299; near Gracemere, *O'Shanesy*.

This is still a very imperfectly known species, so far as the Kew Herbarium is concerned, which is the only one I have access to at the present time. It is the only species known to me with setulose-muricate fruits.

6. *N. angustipetala* (Craib), comb. nov.—*Gynostemma angustipetalum* Craib. *Alsomitra angustipetala* Craib Fl. Siam. Enum. 1: 767 (1931).

Leaflets 5, all separately stalked, unequal-sized, narrowly elliptic to obovate, emarginate and mucronate or cuspidate-mucronate; ripe fruits 5 cm. long; seeds obovate in outline, 8 mm. long, very densely warted all over but not lobulate, with a long terminal wing.

Thailand: *Put* 2650; 3071; 3087; 4109; *Winit* 1422; *Kerr* 19985.

7. *N. pubigera* (Prain), comb. nov.—*Alsomitra pubigera* Prain in Journ. Asiat. Soc. Beng. 67, ii. 292 (1898). 'A. clavigera' Ridl. Fl. Mal. Penins. i. 852. *Hemsleya Henryi* Cogn. in Engl. Pflanzenr. 4, cclxxv. 1: 26 (1916).

Distinguished at once in the genus by its fruits, which are very shortly and softly tomentellous; the shoots and nerves of the leaves are similarly clothed with hairs; seeds as in *N. integrifoliola*.

Upper Burma: Kachin Hills, fl. Nov., *King's Collector* (*Shaik Mokim*); without locality, *Forrest* 13681. Thailand: Me Keng, Oct., *Kerr* 6463. Tachang, Jan., *Kerr* 9869. South Yunnan: Szemao, 1600 m., *Henry* 13375; 13420. Malay Peninsula: various localities, *Curtis* 2504; *Singapore Field No.* 7561; *Henderson* 21388; 29154.

8. *N. integrifoliola* (Cogn.), comb. nov. *Gynostemma integrifoliola* Cogn. in DC. Monogr. Phanerog. iii. 916 (1881). *Alsomitra integrifoliola* Hayata in Journ. Coll. Sci. Tokyo, xxx: Art. 1, 121 (1911), et Ic. Pl. Formos. i: tt. 38–9 (1911); Cogn. in Engl. Pflanzenr. 4, cclxxv. 1: 17 (1916). 'A. integrifolia' Ind. Kew.

Leaflets glabrous, the middle one acutely acuminate and sometimes with a pair of large glands at the base; capsule 5.5 cm. long; seed-body undulate-lobulate and closely warted, with a terminal wing 1.5 cm. long.

Formosa: *Henry* 1556; 1556a; *Wilson* 10017; 10838; *Price* 1075. Philippines: various localities, *Cuming* 517; 767; *Loher* 2126; 2127; 2128; 5110; 6075; 6082; *Elmer* 6694; 17035; 17353; *Merrill* 1527; 4313; *Ahern's collector* 1897; 3409; *Meyer* 2425; *Bur. of Sci.* No. 47046.

Fiji: Bua district, climber on trees and bushes at the edge of forests, *Horne* 1074.

9. *N. tonkinensis* (Gagnep.), comb. nov.—*Alsomitra tonkinensis* Gagnep. in Bull. Mus. Hist. Nat. Paris, xxiv. 372 (1918).

Leaves very sparsely pubescent, the terminal one apparently without glands at the base; leaflets of the inflorescence 5, the 4th and 5th very small.

Tonkin: Hanoi Prov.; Kien-khe, *Bon* 2550; 3551. *Latson*; *Bon* 3062; 3125. Bavi Mt., *Balansa* 4024.

10. *N. plena* (Craib), comb. nov.—*Alsomitra plena* Craib in Kew Bull. 1930: 408.

Leaflets minutely pubescent, those of the female plant much less deeply divided than of the male; fruits 4 cm. long, glaucous; seeds very slightly lobulate and warted, with a rather short terminal wing.

Thailand: various localities, *Kerr* 3037; 9143; 16191; 16191A; *Put* 2496; *Nai Noe* 106; *Winit* 1535.

11. *N. rotundifoliola* (Cogn.), comb. nov.—*Alsomitra rotundifoliola* Cogn., in Engl. Pflanzenr. 4, cclxxv. 1: 13 (1916).

New Guinea: Buragamata, near Namatanai, *Peckel* 471—not seen.

12. *N. sarcophylla* (Roem.), comb. nov.—*Alsomitra sarcophylla* Roem. Synops. ii. 118 (1846); Hook. f. Bot. Mag. t. 6017 (1873); C.B.Cl. in Hook. f. Fl. Brit. Ind. ii. 634 (1879); Cogn. in DC. Mongr. Phan. iii. 929 (1881), et in Engl. Pflanzenr. 4, cclxxv. 1: 13 fig. 3, J-P (1916); Cogn. in DC. Monogr. iii. 929. *Zanonia sarcophylla* Wall. Pl. Asiat. Rar. ii. 28, t. 133 (1831).

Petiole very short; leaflets fleshy, narrowly lanceolate to broadly elliptic, slightly mucronate; fruits 3–4 cm. long; seeds 5–6 mm. long, with a marginal callus extended into two horns at the top on each side, densely verrucose, and with a thin terminal wing.

Burma: Prome, *Wallich* 3724; *Kurz* 1893; *English* 14.

Thailand: various localities, *Kerr* 1625; 17661; *Put* 1384; *Spire* 848; *Collins* 362; 596; 1071; 1412; 2007; *Marcan* 554; *Ridley's* collector 3975.

Philippines: Luzon, *Copeland* 255. Timor, *Teysmann*.

13. *N. philippinensis* (Cogn.), comb. nov.—*Alsomitra philippinensis* Cogn. in Engl. Pflanzenr. 4, cclxxv. 1: 15 (1916). Philippines: Luzon; Lamo River, Bataan Prov., *Copeland* 255—not seen.

14. *N. Muellieri* (Cogn.), comb. nov.—*Alsomitra Muellieri* Cogn. in Bull. Acad. Belg. Ser. 3, xiv. 363 (1887), et in Engl. Pflanzenr. 4, cclxxv. 1: 15 (1916).

New Guinea: Islands to the south east, *Armit*—not seen.

15. *N. trifoliolata* (F. Muell.), comb. nov.—*Melothria trifoliolata* F. Muell. Fragm. v. 181 (1866). *Alsomitra trifoliolata* K. Schum. in Notizbl.



Bot. Gart. Berlin, ii. 155 (1898); Cogn. in Engl. Pflanzenr. 4, cclxxv. 1: 15 (1916). *A. Hookeri* F. Muell. Fragm. vi. 118 (1868).

Leaflets very thin and glabrous, elliptic, very shortly acuminate; seeds pectinate-lobulate, slightly verrucose, with an oblique oblong membranous terminal wing.

New Guinea: various localities, *Hellwig* 673; *Warburg* 20915; *Beccari*; *Parkinson* 82. N. Australia: Cape York, *Hann* 362; Rockingham Bay, *Mueller*.

16. **N. Balansae** (Gagnep.), comb. nov.—*Alsomitra Balansae* Gagnep. in Bull. Mus. Hist. Nat. Paris, xxiv. 371 (1918).

Tonkin: Cho-bo, Rivière Noire, *Balansa* 4022. Vo-xa, in the Thung-dang mountains, *Bon* 3247.

17. **N. suberosa** (F. M. Bail.), comb. nov.—*Alsomitra suberosa* F. M. Bail. Syn. Queensl. Fl. Suppl. ii. 28 (1888); Cogn. in Engl. Pflanzenr. 4, cclxxv. 1: 18 (1916).

Leaflets subequal, the middle one broadly lanceolate, undulate-denticulate, the lateral ones obliquely ovate, glabrous.

Australia: Queensland; Euoggera, Aug., *Bailey*; Samford Range and Mt. Glorious, very common on edge of rain forest, Nov., *White* 9647.

18. **N. Beccariana** (Cogn.), comb. nov.—*Alsomitra Beccariana* Cogn. in DC. Monogr. 3: 932 (1881), et in Engl. Pflanzenr. 4, 275, 1: 15 (1916).

New Guinea: Key Island, *Beccari*.

19. **N. pubescens** (Merr.), comb. nov.—*Alsomitra pubescens* Merrill in Philipp. Journ. Sci. Bot. xiii. 64 (1918).

Leaflets rhomboid-elliptic, broadly acuminate, with 2 linear glands near the base, softly puberulous on both surfaces; lateral leaflets with a very small lobule at the base.

Philippines: Luzon (*Mabesa* For. Bur. no. 26346).

20. **N. Schefferiana** (Cogn.), comb. nov.—*Alsomitra Schefferiana* Cogn. in DC. Monogr. iii. 932 (1881), et in Engl. Pflanzenr. 4, cclxxv. 1: 16 (1916); incl. var. *minor* Cogn.

Celebes: Pangkadjena, *Teysmann*. New Guinea: Namatanai, *Peckel* 328.

I have seen only a fruiting specimen without leaves; the inflorescence is softly tomentose, and the rather small fruits (2.5 cm. long) glabrous.

21. **N. clavigera** (Roem.), comb. nov.—*Alsomitra clavigera* Roem. Synop. ii. 118 (1846); C.B.Cl. in Hook. f. Fl. Brit. Ind. ii. 634 (1879); Cogn. in DC. Monogr. iii. 931 (1881), et in Engl. Pflanzenr. 4, cclxxv. 1: 16 (1916). *Zanonia clavigera* Wall. Pl. Asiat. Rar. ii. 28 (1831). *Z. integerrima* Wall. ex. Cogn. 11. cc., nomen.

Leaflets acutely acuminate, the middle one biglandular at the base, the lateral ones often with one gland on the peripheral side; fruits 7-9 cm. long;

seeds deeply lobulate and warted, with a terminal membranous wing 2 cm. long.

Sikkim, *Treutler* 710; *Clarke* 27042; *Gamble*.

Assam: Khasia Hills, *Hooker & Thomson* 2160; Sillet, *Wallich* 3725. Tenasserim, *Helfer* 2520.

Burma: Mergui distr., *Parker* 2450. Garo Hills, *Parry* 1008.

Philippines: Luzon, *Loher* 2129; *Vidal* 2908.

22. **N. Stephensiana** (F. Muell.), com. nov. *Zanonia Stephensiana* F. Muell. *Fragm.* viii. 181 (1874). *Alsomitra Stephensiana* Cogn. in DC. *Monogr. Phanerog.* iii. 934 (1881), et in *Engl. Pflanzenr.* 4, cclxxv. 1: 17 (1916).

Eastern Australia: Barnard Island, *W. Hill*—not seen.

#### *Excluded species*

*A. peruviana* Huber = **Siolmatra peruviana** (Huber) Cogn. *A. braziliensis* Cogn. = **Siolmatra braziliensis** Cogn. (Baill.). *A. pedatifolia* Cogn. = **Siolmatra pedatifolia** (Cogn.) Cogn.

#### SUMMARY

Evidence is brought forward that certain Indo-Malayan Cucurbitaceous plants with beautifully winged seeds hitherto known as *Macrozanonia* should be called **Alsomitra**, since *M. macrocarpa* (Blume) Cogn. is the type species of that genus as originally established by Roemer. An emended description is provided to include a second imperfectly known species from the Malay Peninsula.

For the remainder of the species referred by various authors to *Alsomitra*, the new name **Neoalsomitra** is proposed. A key to the species under the necessary new combinations is provided and an enumeration indicating their distribution.

# The Mycorrhizal Relations of Larch

## III. Mycorrhiza Formation in Nature<sup>1</sup>

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With Plate II and six Figures in the Text

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### I. INTRODUCTION

EUROPEAN larch (*Larix decidua* Mill.) is planted in considerable quantity in the British Isles; although it is not native to these islands it has long been established in some localities. Nevertheless, it may be anticipated that many of the fungi with which it forms mycorrhizal associations in its native habitat will not be available in the soils of this country. *Boletus elegans*, which is known to be a mycorrhiza-former with larch, does occur in plantations in this country but is probably absent from many sites. Now there is abundant evidence from various parts of the world that exotic trees may fail to establish themselves in otherwise suitable habitats owing to the absence of their normal endophytes (Hatch, 1936); often no success is possible until the soil has been inoculated with the appropriate fungi. In other cases, though mycorrhizal associations are formed, no balanced condition is reached and the health of the trees is imperilled. This aspect of tree culture has been emphasized by Rayner (1939), who shows how important it is for forestry practice to have some knowledge of the range of mycorrhizal structure found in such exotic conifers and some estimate of what constitutes a balanced condition. The present paper attempts to supply this need in the case of larch raised in the British Isles.

There are no recent detailed descriptions of the mycorrhizal conditions of mature European larch trees in their native habitats. McDougal (1914)

<sup>1</sup> Part of a thesis approved for the Degree of Doctor of Philosophy in the University of London.

published a description of root infection in mature larch in the U.S.A.; Melin (1922) described the conditions in six-year-old trees in a forest nursery in Sweden; but in neither case do the conditions described correspond to anything observed by the writer. Laing (1923, 1932) considers that normal larch mycorrhizas from British habitats can be classified into three types, the ectotrophic, semi-endotrophic, and endotrophic. But in the absence of any information as to soil conditions, age of the trees, detailed structure of the mycorrhizas or evidence of the specific nature of the endophytes concerned, these observations are of small value for comparative purposes. Moreover, it is possible that both the semi-endotrophic and endotrophic types result from various abnormal conditions.

## II. GENERAL FEATURES OF THE ROOT SYSTEM

A number of conifers, notably species of pine, have been shown to have two types of root, polyarch long roots and diarch or monarch short roots, alternatively termed laterals and sublateral roots; it is the sublateral roots which normally become mycorrhizas. Aldrich-Blake (1930), working with Corsican pine roots, measured the diameters of circles drawn through the protoxylems in transverse sections taken at the point of emergence of the root, and he found that the values obtained could be grouped into two classes. The so-called 'long' roots had protoxylem diameters above a certain value, the 'short' roots had diameters below this value; so it seemed clear that the two types of root had differed from the beginning. Hatch and Doak (1933) found in pure culture seedlings of *Pinus Strobus* that sublateral roots were produced which remained short though uninfected, and they concluded that mycorrhizas remained short because they were a morphological entity and not because the enwrapping fungus prevented further growth, as was believed at one time (McDougal, 1914; Masui, 1927).

Casual observation of the root system of larch does not reveal any clear differentiation into long and short roots, since the mycorrhizal branch systems often reach considerable length, and all roots, except the monarch ultimate branches of the mycorrhizas, are diarch whatever their size. However, it seemed worth while to apply Aldrich-Blake's method to this species. Slender roots which showed no evidence of becoming infected and others which were already mycorrhizas were sectioned at the point of emergence from the mother root and the protoxylem diameters were measured. Two classes were clearly distinguishable. The mean protoxylem diameter of 16 infected roots was  $117 \pm 23.3 \mu$ , of 16 uninfected roots  $243.2 \pm 50 \mu$ ; the probability of significance of the difference between these two measurements was over a 100 to 1. There is no doubt that the uninfected roots were true 'long' roots and the infected ones were 'short' roots. Occasionally infected roots showing monopodial branching are found to be long roots as determined by protoxylem size, but the structure of the mycorrhiza thus formed is different from that in infected short roots, and it only serves to emphasize the difference between the two



root types (see section V). It is these short roots which normally form mycorrhizas. They are easily distinguished macroscopically when fully developed; for infection results in the repeated formation of rows of branches at right angles to the main axis of the short root to give a flat monopodial branched system (see Pl. II, Fig. 1). While in the writer's knowledge this typical branch system never occurs apart from infection, infection does not always result in monopodial branching. Where infection occurs without monopodial branching the internal structure always exhibits other non-typical features, such as intracellular infection and absence of a tannin layer, which may be regarded as marks of pseudo-mycorrhizal infection (Rayner, 1934). The fact that, in general, repeated monopodial branching follows infection disposes finally of the suggestion that the presence of the fungal mantle exerts a restraining influence on the growth in length of the sublateral roots, thus giving rise to short roots. While superficially that might appear to be the case in pine, where the infected roots are short, in larch mycorrhizas they are often 1.5 cm. long and have evidently grown and branched vigorously since first infected. In view of these conditions in larch the term sublateral rather than short root will be used throughout the remainder of this paper.

### III. FUNGAL CONSTITUENTS AND THEIR ISOLATION

Melin (1925 and 1925 *a*), Hammerlund (1923), Peyronnel (1920, 1921, 1922 *a* and *b*, 1929), and Laing (1932) all produce evidence that larch can form mycorrhizal associations with a number of different species of fungi. In the present inquiry evidence of infection by a great many different species, most of which remain unidentified, has been obtained from the examination of the hyphal strands and fragments of hyphae attached to the mantle in infected roots. In three cases successful isolations from roots have established infection by *Boletus elegans*, *Paxillus involutus*, and a black mycelium (see below). In addition, three or four distinct hyaline clamp-mycelia have been observed to form well-balanced mycorrhizas and a few yellow and yellow-brown hymenomycete mycelia can also infect the roots, though the latter are probably deleterious in their effects. A number of the types observed may be casual infections, but some occur with great regularity in many different habitats and with essentially similar morphological effects on the root. In any given locality a number of different infections can usually be found within a very small area, so that any one tree is in association with more than one fungus. Naturally sown seedlings in which it has been possible to examine all the roots have shown three or more different infections on one plant, as if the area inhabited by any one fungus is small and the roots spreading through the soil become infected by many different fungi living in pockets in the soil. This haphazard nature of the infection is very marked in some localities.

Melin (1922) attempted unsuccessfully to isolate *B. elegans* from larch mycorrhizas which were assumed to be infected by this fungus; he used the mercuric chloride method of sterilization which had proved successful with

*Pinus sylvestris* and *Picea Abies*. He regarded his failure to isolate the fungus as due not to excessive sterilization but to the loss of the power of independent growth on the part of the endophyte. The present writer's experience, however, proves conclusively that *B. elegans* does not become incapable of growth after entry into the root; it must be concluded that Melin's sterilization was in fact too severe.

#### *Isolation of Boletus elegans* Schum.

Over a four-year period repeated attempts were made to isolate *B. elegans* from larch mycorrhizas using sterilization by mercuric chloride. Eventually success was obtained with this and another method. The first method used was that of Ternetz (1907); mycorrhizas collected from the vicinity of sporophores of *B. elegans* were washed repeatedly with sterilized distilled water, placed in 1 per cent. hydrochloric acid for 60 secs., and after washing once more in sterilized distilled water were plated on Melin's glucose. All the cultures were considerably contaminated, but three cultures also gave rise after four days to a slow-growing, densely tufted, white mycelium which rapidly turned yellow and then orange-brown. This appearance, combined with the fact that papillation of the hyphae and 'paarige' branching were observed during microscopic examination, left little doubt that the mycelium was that of *B. elegans*. Further material similar to that used above was sterilized by washing in 0.1 per cent. mercuric chloride for less than 20 secs. and then repeatedly in sterilized distilled water, it was then plated on Melin's glucose. Twenty-three cultures remained sterile; one culture, however, had a slight bacterial contamination and twenty-one days after inoculation developed a white, densely tufted mycelium which turned yellow and then brown; it proved possible to subculture this without contamination. Presumably this one root escaped the full effects of the sterilizing agent as shown by the bacterial contamination and in consequence the endophyte remained alive. It was a matter of chance that on this occasion success was obtained with the mercuric chloride method which is normally too drastic for the isolation of *B. elegans*.

The evidence that the mycelium isolated on these two occasions is *B. elegans* can be summarized as follows; (i) For over two years cultures of the endophyte have been grown side by side with cultures of *B. elegans* obtained from sporophore tissue. The two fungi are indistinguishable macroscopically and behave similarly on all media. The rate of growth of the endophyte has increased slowly during this time and now is only slightly less than that of the cultures isolated from sporophore tissue. (ii) Microscopic examination reveals that the two mycelia differ in no respect. Both have papillated hyphae 2–3  $\mu$  wide, 'paarige' branching, brown hyphae, and compound hyphal strands (see How, 1940, for detailed description of the mycelium of *B. elegans*).

The evidence that the fungus isolated was the true endophyte is as follows: (i) When the mantles of the roots actually giving rise to the endophyte were compared with others from the same material, they were found to be similar in

structure and from the latter papillated hyphae radiated. (ii) The hyphal strands in the mycelium around these mycorrhizas were compound and similar in structure to those of *B. elegans* in pure culture; therefore this species was undoubtedly present in the vicinity of the root. (iii) This type of mantle structure has been observed many times in many localities and always sporophores have been present in the vicinity.

While it is realized that the only absolute proof of identification would be the production of sporophores of *B. elegans* by the endophyte in pure culture and the development of mycorrhizas similar to those from which it was isolated as a result of the inoculation of pure culture seedlings; nevertheless, familiarity with the fungus in pure culture leaves no doubt in this case that the endophyte is *B. elegans*.

#### *Isolation of Paxillus involutus* Fr.

Larch mycorrhizas were sterilized for 30 or 60 secs. with 0.1 per cent. mercuric chloride, washed repeatedly with sterilized distilled water, and plated on beerwort agar. Most of the cultures remained sterile; two, however, developed red-brown colonies of a mycelium bearing clamp connexions. Examination of mycorrhizas exactly similar to those used in the isolation showed that the mantle was formed by hyphae with straw-coloured walls and clamp connexions. The mycelium was kept in culture for some time before its identity was established. A culture of *Paxillus panuoides* Fr. supplied by the Forest Products Research Laboratory, Princes Risborough, Bucks., somewhat resembled the brown endophyte. It was therefore thought possible that the fungus might be a terrestrial species of *Paxillus*. The only species of this genus known to occur in the plantation from which the roots had been collected was *P. involutus*, which was found abundantly. Accordingly, tissue cultures were made from young sporophores of this species. These were brown in colour, though paler than the endophyte; in general appearance and cultural behaviour the two fungi were similar, particularly in their preference for an acid culture medium (see Table I). Microscopic examination revealed that both fungi had hyphae with straw-coloured walls, 1.5–4.5  $\mu$  wide, and very frequent clamp connexions. Again complete proof is lacking, but there is a very high probability that this endophyte is *P. involutus*.

TABLE I

#### *Comparison of the Reactions to Acidity of P. involutus and an Endophyte*

Substrate	<i>P. involutus</i>	Endophyte
(1) 2% malt agar	Heavy browning of substrate. Growth not so good as (2).	Heavy browning of substrate. Growth not so good as (2).
(2) 2% malt agar plus 0.5% malic acid	No colour excreted, growth very good.	No colour excreted, growth very good.
(3) 2% malt agar plus 1% malic acid	No colour excreted, growth similar to (1).	No colour excreted, growth very slow.



*Isolation of a 'black' mycelium.*

Some dark brown roots obtained from Scotland were sterilized by the Ternetz method. They gave rise to pure cultures, which were green-grey in colour at first, later greenish-black, and finally dark brownish-black; at this stage the mycelium forms a hard black crust over the surface of the substrate, no pigment is excreted into the medium. The hyphae are  $1.5-3.5\ \mu$  wide, hyaline in colour when young, later becoming dark brown. The walls of some hyphae have granular excretions giving a papillose appearance, which, however, is quite distinct from that shown by hyphae of *Boletus* spp.; there are no clamp connexions. The apices of some of the aerial hyphae become coiled into a ring. Thin mycelial strands occur  $7-9\ \mu$  in width. Strings of spore-like bodies are formed by the substrate hyphae and these hyphae are often terminated by a long tapering filament. Sclerotia are formed on poor media such as distilled water agar.

This fungus is clearly of the same type as Melin's *Mycelium radidis atrovirens*, isolated from roots of *Pinus sylvestris* (1923), since it differs in no way from the recorded description of that mycelium. It has since been isolated again from larch roots collected in south-east England; these cultures are identical with the Scotch cultures. Moreover, its behaviour in pure culture, e.g. its attack on filter-paper cellulose, indicates that physiologically it is a member of the 'atrovirens' group of soil fungi.

## IV. THE STRUCTURAL FEATURES OF SOME INFECTIONS

It has been possible to distinguish over a dozen types of infection by using the characters of the mantle, supplemented by any information as to the nature of the fungus which may be deduced from the fragments of the hyphae available. The following seven types have been chosen for description, either because the nature of the endophyte has been more or less definitely established or because the type occurs very frequently and is probably therefore not a casual infection.

All the material examined was first fixed in Doak's solution (How, 1941) and then cut by hand. The descriptions record the appearance of sublateral roots in transverse section.

1. *Endophyte: Boletus elegans* Schum. (Pl. II, Fig. 2).

This is a widely distributed form in England. The mycorrhizas here described are similar to those from which *B. elegans* was isolated. The mycorrhizas are pinkish-brown in colour and monopodially branched; the branches are irregularly spaced a small distance apart and the apices are pointed. The hyphae radiating from the mantle are numerous, hyaline, thin-walled, papillose, and  $1.5-3.0\ \mu$  wide; there are no clamp connexions. The mantle has a rough margin and is  $15-60\ \mu$  in width. When wider than  $20\ \mu$  it consists of two layers, an outer layer of hyphae woven loosely in a direction transverse to



the root axis and an inner layer enclosing 0 to 3 layers of dead cortical filaments with the hyphae tightly woven in a longitudinal direction. When below  $20\ \mu$  in width only the inner layer is present; in no case are the hyphae swollen, nor has a pseudo-parenchymatous effect been observed. The tannin layer is one cell deep, composed of tannin-containing cells, granular cells, and unmodified cortical cells. The Hartig net extends over the entire outer cortex, the cortical cells are oblong in shape, the walls between them are expanded by the fungus to  $2.5\ \mu$  in width. No intracellular infection has been observed.

There is no evidence from pot cultures or from growth in the field as to whether this type of mycorrhizal infection is accompanied by good growth on the part of the tree. In the absence, however, of any signs of the histological features known to indicate the presence of deleterious conditions in pine, it may be assumed that a structure of this type is the expression of a balanced mycorrhizal relationship.

## 2. *Endophyte: B. viscidus* L.?

This type has only been collected from one locality in the vicinity of the sporophores of *B. viscidus*, a species reputed to grow exclusively in larch plantations. The identity of the endophyte as *B. viscidus* is by no means certain, but it is rendered probable by the fact that the mycelium below the sporophores is pinkish-white in colour and so are the mycorrhizas; also the hyphae below the sporophores have 'paarige' branching and papillation, as do the hyphae in the vicinity of the mycorrhizas; and those radiating from the mantle are also papillated. Lastly the structure of the mycorrhizas is reminiscent of the *B. elegans* type though different, as might be expected if another species of *Boletus* was concerned.

The mycorrhizas are monopodially branched, though the branching is somewhat irregular and the apices are pointed. The hyphae radiating from the mantle are few in number, hyaline, papillose, and  $2-4\ \mu$  in width. The mantle has a very rough margin thickly covered with large refractive granules; it varies from 12 to  $80\ \mu$  in width and consists of a single homogeneous layer of transversely woven hyphae. The tannin layer is one cell deep, composed of tannin containing cells and unmodified cells; the next cell layer consists of granular cells. The Hartig net extends across the entire outer cortex; many of the outer cortical cells are oblong in shape, and the walls between them are expanded by the fungus to a width of  $1.5-4.5\ \mu$ . No intracellular infection has been observed.

There would be no reason for assuming that this type was anything but a balanced association were it not for the large quantities of granules on the outside of the mantle. This condition may indicate the existence of some abnormal physiological state similar to that which produces the 'messy' type in pine (Rayner, 1939); but more experience would be necessary to confirm this. It is possible that the presence of granules is a constant feature of infection by this species, which normally forms a balanced relationship with the tree.

3. *Endophyte: Paxillus involutus* Fr. (Pl. II, Fig. 3).

This type has only been observed once with certainty. The mycorrhizas here described are similar to those from which *P. involutus* was isolated. The mycorrhizas are dull brown in colour, with monopodial branching at infrequent intervals and pointed apices. The hyphae radiating from the mantle are numerous, pale straw-coloured with rather thick walls; they are  $1.5-3.5\ \mu$  in width and have frequent clamp connexions. The mantle, which has a rough margin, is  $14-36\ \mu$  in width, pale brown in colour, and composed of loosely woven hyphae; these are woven in all directions with a tendency to run in a transverse direction near the outer margin. The tannin layer is one cell deep, with a few non-tannin cells; no granular cells have been observed. The Hartig net extends across the entire outer cortex, many of the cells are oblong, and the walls between them are swollen by the hyphae to a width of  $3-6\ \mu$ . There are traces of intracellular infection of the haustorial type. In old mycorrhizas the walls between the cortical cells are even more swollen and contain more than one strand of hyphae, so that the cells appear to be isolated in a pseudoparenchymatous tissue. This and the intracellular infection are the only histological indications that infection by *P. involutus* may not give rise to a balanced relationship.

4. *Endophyte: unknown.*

This is a widely distributed form, and as such is of interest even though there is no clue as to the endophyte concerned. The mycorrhizas are pinkish-brown in colour, either unbranched or irregularly branched; the apices are very rounded. The mantle is completely smooth on the outside, hyaline in colour, and therefore formed by a hyaline fungus; it is  $25-65\ \mu$  in width and parenchymatous in structure, with an outer layer of wide brick-shaped elements and an inner layer of tightly woven narrow elements. The tannin layer is only one cell deep and composed of flattened tannin cells with a few granular cells. The Hartig net extends across the outer cortex irregularly; often it does not reach the inner cortex. The cells are isodiametric and the walls between them when penetrated by the net are  $1.5-3.5\ \mu$  wide. There is no intracellular infection.

The absence of true monopodial branching, the very thick mantle, and poorly developed Hartig net are all features which suggest that this type of infection is of doubtful value.

5. *Endophyte: A yellow hymenomycete mycelium* (Pl. II, Fig. 4).

This is another very common infection. Usually the infected roots are brown in colour, a trifle swollen at the tip and unbranched. They are obviously attacked by a hymenomycete mycelium as shown by the clamp connexions on the encircling hyphae; these are greenish-yellow in colour and  $1.4-2.2\ \mu$  wide. No proper mantle is formed, the hyphae are loosely grouped round the root. There is no true tannin layer but only one or two layers of flattened cortical filaments. The Hartig net is well developed, extending across the outer

cortex, the cortical cells are isodiametric, and the walls between them are  $1.5-3.0\mu$  in width. There is no intracellular infection.

The absence of mantle and tannin layer suggests that this association is of doubtful value, though the Hartig net formation is normal.

#### 6. *Endophyte: Mycelium radialis atrovirens* (Pl. II, Fig. 5).

This is the type from which *M. r. atrovirens* was isolated; roots infected by this fungus have not been found frequently, though the mycelium appears to be present in many soils. The infected roots are black in colour and monopodially branched at infrequent intervals. The hyphae which are occasionally found attached to the mantle are  $1.5-4.0\mu$  in width; they are green-brown in colour and have rigid walls without clamp connexions. The mantle is  $34-40\mu$  in width; the outer layer is dark brown, due to the deposition of a brown pigment in the walls of the hyphae which are closely woven and run in a transverse plane; the inner layer close to the tannin cells is straw-coloured and the hyphae run in a longitudinal direction. The tannin layer is one cell in depth, with an occasional granular cell. The Hartig net is very uneven in depth, sometimes extending to the inner cortex, often only involving one or two layers of the outer cortex. The cortical cells are mainly isodiametric, the walls between them being  $2.2-6.5\mu$  wide and pale brown in colour, due to the intercellular hyphae. Intracellular infection of the haustorial type occurs frequently and often hyphae are found to be growing straight through the cells.

While the condition of the tannin layer and Hartig net bear some resemblance to that found in normal associations the following considerations point to the conclusion that this is a deleterious type of infection. Firstly, no mycelium of 'atrovirens' type grown by Dr. I. Levisohn in this laboratory has ever been observed to be other than a pseudo-mycorrhiza-former with pine and spruce. Secondly, there is no evidence in the literature that *M. r. atrovirens* is capable of forming a balanced association with any species. Thirdly, the parasitic behaviour of the strain of *M. r. atrovirens* isolated from larch as compared with that of *B. elegans* is shown by some observations which were made on pure culture larch seedlings inoculated with either *B. elegans* or this strain of *M. r. atrovirens* (for the method of culture see Appendix). After  $2\frac{1}{2}$  months the seedlings inoculated with *B. elegans* showed a slight intercellular penetration of the cortex, but those inoculated with *M. r. atrovirens* were subject to a strong infection of the intracellular type. It may therefore be inferred that it is improbable that *M. r. atrovirens* can form a beneficial association with larch roots, but no final conclusion can be drawn until the effect of such association on the health of the whole plant has been studied in experimental culture under a wide range of conditions.

#### 7. *Endophyte: a 'black' mycelium.*

This type has been chosen because it is an example of the undoubted pseudo-mycorrhizal condition in larch. The roots are unbranched with black swollen



tips, from which radiate stiff dark brown hyphae; these are  $1.5-2.0\ \mu$  wide and have no clamp connexions. The mantle varies from  $15$  to  $35\ \mu$  in width; it is composed of a single layer of dark brown hyphae woven tightly together and running in all directions. The tannin layer is poorly developed and there are no granular cells. The Hartig net occurs only sporadically and then it is but one cell deep; the cortical cells are isodiametric with very thin walls,  $0.75-1.5\ \mu$  in width. There is no intracellular infection.

#### V. GENERAL FEATURES OF MYCORRHIZA FORMATION

In addition to the seven types described in detail above, many other types of mycorrhizal infection have also been examined. It is therefore possible to give a general account of the variations in histological structure resulting from the infection of larch roots in trees raised in the British Isles. In this general description no account has been taken of mycorrhizal structure in seedlings, since there is evidence that this differs somewhat from that occurring in mature trees; but there is not sufficient information available to determine the exact extent of the difference.

##### *Mantle.*

The structure of the mantle depends entirely on the fungus composing it, the type of weaving being a constant feature for any one fungus. The outer margin is usually smooth with occasional hyphae radiating from it; however, in one attack by an unidentified fungus, the outside of the mantle was covered by a single layer of flasked-shaped fungal cells, reminiscent of cystidia. Peyronnel (1921) reported a similar condition in larch mycorrhizas in Italy, and attributed it to infection by a species of *Russula*. The width of the mantle may be dependent on the age of the mycorrhiza or the edaphic conditions. In general the mantle increases in width towards the base of a mycorrhiza; most fungal constituents form mantles between  $10\ \mu$  and  $70\ \mu$  in width.

##### *Tannin layer.*

This layer, situated where mantle and cortex meet, is usually at least one cell in depth in typical mycorrhizas; the only variations occur in the infection of a lateral root by a true mycorrhizal fungus or the pseudo-mycorrhizal infection of a sublateral; in these cases the tannin layer is often absent. Transverse sections of normal mycorrhizas often show further layers of tannin cells outside the tannin cells proper; these are flattened and surrounded by the mantle which appears to have partially absorbed the cells in a parasitic manner. Laing (1932) stated that the fungus actually destroys the cell and thereby eats into the root; but there is no evidence for the occurrence of such parasitism. The examination of serial transverse sections of roots from the apex backwards indicates that these flattened cells are often the remains of cortical filaments which serve as root-hairs (see Melin, 1922, for a detailed description of these structures). In uninfected roots the cells of these filaments



which become stained yellow-brown are sloughed off; in infected roots they become surrounded by the mantle before they can be torn off and in consequence become incorporated in it. In some lateral root infections where the mantle does not cover the tip, the layers of flattened cortical cells are missing from the mantle, since these had been sloughed off before the mantle enveloped the outside of the root. The examination of longitudinal sections of mycorrhizas tends to confirm this view of the origin of these outer cell layers in the mantle; the position of the tannin layer proper in relation to the other cortical cell layers does not alter throughout the length of the root but remains fixed so that the tannin layer is continuous with the suberin sheath over the meristem itself; moreover, in sections stained with Delafield's haematoxylin the tannin cells proper are dark purple in colour; the flattened cortical filaments are golden brown. It has often been supposed that the production of a tannin layer is the response of the cortical cells to their isolation by the encroaching fungus; if this is so, it is difficult to see why the cortical cells of the Hartig net which are also completely surrounded by fungal hyphae should not respond in the same manner. Probably the tannin layer corresponds merely to the outer protective layer of dead cortical cells surrounding uninfected roots and has no particular relation to fungal infection. The true tannin layer usually consists of cells filled with tannin and empty cells with thickened brown walls; occasionally unmodified cells are included. In pine mycorrhizas Melin (1923) noted two or three layers of cells filled with granules of varying size inside the tannin layer. In larch mycorrhizas only one layer of granular cells is present at most, and often only two or three such cells are visible in a transverse section, where they occur either incorporated in the tannin layer or just inside it.

#### *Hartig net.*

Within the tannin layer the cortex of a larch root is clearly distinguishable into two parts, an outer two or three layers of cells with unmodified cellulose walls and an inner two layers of cells with cellulose walls much thickened and yellow-brown in colour; the exact nature of this modification is not known; the walls are certainly neither suberised nor lignified. The entry of the fungus between the root cells to form a Hartig net never proceeds into this inner cortex; it is therefore probable that the modification of the walls acts as a barrier. Usually all the cells of the outer cortex are involved in the Hartig net and are thereby at least partly isolated from one another by one layer of hyphae lying between the cell-walls. Occasionally more than one layer of hyphae penetrates between the cells, which are then completely isolated and appear as islands in a pseudo-parenchymatous tissue. This occurs most frequently in old mycorrhizas and is doubtless a sign either of weakness on the part of the root or of the high virulence of the fungus.

In some of the types of infection examined, notably the mycorrhizas formed by *B. elegans*, the cells of the outer cortex in transverse section in the sub-lateral roots are roughly oblong in shape, the radial walls being longer than the

tangential; this gives a very characteristic appearance in transverse section. If, however, uninfected sublateral roots are examined no such appearance is observed, nor is it found in lateral roots which have become infected; the differential behaviour of infected lateral and sublateral roots being particularly marked.

There is no doubt that typically the uninfected root has outer cortical cells which are roughly isodiametric in the transverse plane. There are three possible ways in which this shape could be altered to give a cell of greater width in the radial rather than the tangential plane. Either the square cells could divide by formation of radial walls, so that the tangential walls were bisected, or the presence of the fungus might cause the cells to be compressed radially or both factors might operate together. An attempt was made to determine in which of these ways the oblong cells are actually formed.

The evidence that it is radial division rather than compression which determines the shape of the cells is as follows:

TABLE II

*Relation of Number of Cortical Cells to Type of Root Infection*

Type of infection.	Not infected.	<i>B. elegans</i> (type 1).	Yellow hymeno- mycete mycelium (type 5).	<i>Mycelium radicis atrovirens</i> (type 6).	2nd Black mycelium (type 7).
Number of cortical cells	43	68	45	49	38
	42	59	38	37	32
	41	65	37	44	36
	42	56	36	54	42
	43	96	38	36	49
	43	61	51	47	51
	38	58	39	33	30
	37	63	51	58	34
	37	60	37	50	38
	45	58	42	36	37
Mean (with S.E.)	41.1 ± 2.81	61.7 ± 4.43	41.4 ± 5.72	44.4 ± 8.48	38.7 ± 5.89

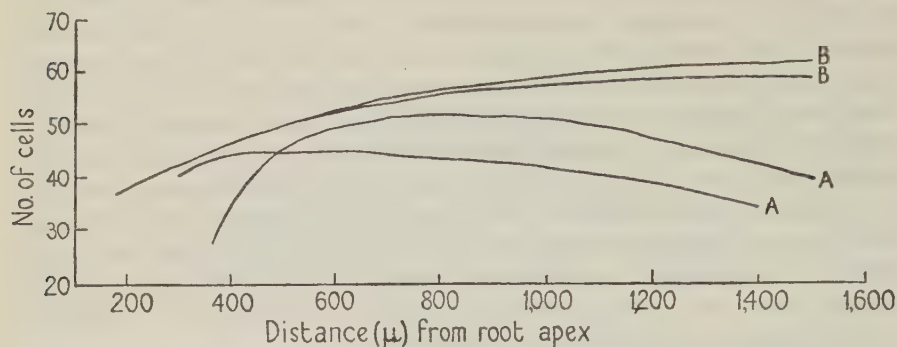
(i) Statistical comparisons (Table II) were made of the number of cells in the fourth cortical row outside the endodermis in various types of sublateral root. This particular cell row was chosen because it was half-way between the thickened inner cortex and the somewhat irregular tannin layer. Unfortunately uninfected sublateral roots are rarely found in nature so that it was difficult to obtain enough examples for comparison. But sublateral roots infected by yellow or black mycelia (i.e. types 5, 6, or 7) are more common and these were also compared with sublateral roots known to be infected by *B. elegans* (Table II). There is considerable variation in the numbers within any type since hand sections cannot be cut at exactly similar levels and the roots vary somewhat. However, the results clearly indicate that the sublateral roots infected with *B. elegans* have more cells in the fourth cortical row than any other

type examined and that this difference is highly significant (Table II). The values for the uninfected roots are probably somewhat too high, owing to the impossibility of being certain that every root is a true sublateral; the lateral roots compared with the sublateral roots have a greater number of cells in each cortical layer, and the inclusion of any such roots would raise the mean values. The importance of the low values obtained from types 5, 6, and 7 lies in the fact that these attacks are extremely unlikely to be followed by a diminution in the number of cortical cells laid down by the growing-point since the protoxylem diameters remained the same; therefore the number of cortical cells which would have been obtained had the same roots remained uninfected cannot be larger than the actual number counted in roots attacked by yellow or black fungi, and it might well be smaller if division occurred in these roots also. The fact that counts from such roots give similar numbers to those obtained from uninfected roots suggests that in all probability no increase in cell number takes place as the result of attack by such fungi. But there is no doubt that counts of root cells support the contention that radial division of cortical cells occurs in roots infected with *B. elegans*.

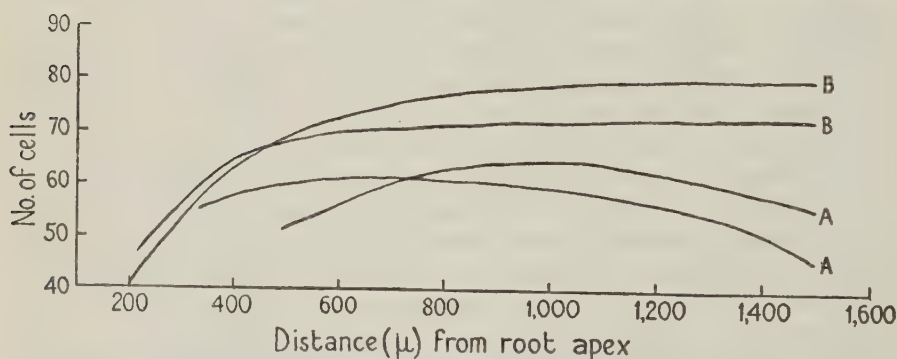
(ii) Two samples of material were collected from different localities; each contained some uninfected sublateral roots and some infected by *B. elegans*. After embedding in paraffin wax transverse sections  $10\ \mu$  thick were cut by microtome from each type of root; the number of cells in the fourth cortical row was counted in certain of the sections taken at frequent intervals from the apex to the base of each root. The variation of cell number with distance from the apex is shown in Text-figs. 1 and 2. It is important when comparing the cell numbers of single roots, rather than the mean values of many roots, to use roots taken from the same small area since variation in soil, &c., may lead to marked differences in the potential number of cortical cells; for example, sample 1 tended to have fewer cortical cells than sample 2. But in each sample the uninfected roots show a tendency for the number of cells to increase up to a certain distance from the apex, after which the number remains steady and may finally decrease. This is a reflection of the life-history of the root, in which a certain amount of radial division of the cortical cells laid down by the meristem occurs just behind the apex and then no further division occurs; the decrease in the number of cortical cells in the basal portion results from the slight increase in the size of the growing-point after emergence from the parent root. In the infected root, however, the number of cells increases to a much higher level behind the growing-point than in uninfected roots, and it remains at that level showing no decrease at the base. Since only roots with similar protoxylem diameters were compared it is legitimate to assume that the potential number of cortical cells was similar in every root, in which case it is clear that more divisions occurred immediately behind the growing-point in the infected than in the uninfected roots. The fact that in the former there is no falling off in cell number towards the base further emphasizes the much greater activity of cell division, which appears to continue



for a greater distance behind the meristem and completely to mask any trace of the smaller number of cortical cells laid down on the first emergence of the root. Had more root material been available the effect of individual variations could have been lessened by the use of mean values; nevertheless, this method gives further proof that increased cell division occurs in infected roots.



TEXT-FIG. 1. Variation in cortical cell number with distance from the root apex in sample 1. A, uninfected sublateral roots; B, sublateral roots infected with *B. elegans*.



TEXT-FIG. 2. Variation in cortical cell number with distance from the root apex in sample 2. A, uninfected sublateral roots; B, sublateral roots infected with *B. elegans*.

(iii) It was noted that the diameter of infected roots, excluding the mantle, was not markedly greater than that of uninfected roots; any cell division which occurs in the radial plane must therefore divide the existing cells so that the tangential walls are halved and the radial walls remain the same. If the oblong shape of the cells in the infected roots is due to such division rather than to compression the increase in the number of oblong cells in the infected roots compared with the uninfected will be twice the difference between the total number of cells in the two types of root. If the difference is more than twice, then the shape of some oblong cells is determined by compression rather than division.



Camera lucida drawings were made of the fourth cortical cell row in one section ( $1,600\ \mu$  from the apex), from each of the serial sections used previously. The radial and tangential axes of each cell were measured and the number of oblong, isodiametric, and tangentially elongated cells was estimated. An 'oblong' cell was defined as one having the radial axis at least  $2.5\ \mu$  longer than the tangential and an 'elongated' cell as one having the radial axis at least  $2.5\ \mu$  shorter than the tangential. In order to reduce the effect of individual variations the cell numbers of four roots are considered together in each case. Table III shows that the ratio of the differences between the total number of cells of all types and the number of 'oblong' cells is less than two, viz.  $1:1.9$ . Thus division of cells would account for the number of oblong cells without assuming that compression of the cells had also occurred.

TABLE III

*Numbers of 'Oblong' Cortical Cells in 4 Uninfected and 4 Infected Sublateral Roots of Larch*

	Uninfected.	Infected.	Difference.	Ratio of differences.
Total number of cells	33	68	—	—
	27	62	—	—
	58	78	—	—
	67	81	—	—
	185	289	104	1.9
Number of oblong cells	9	64	—	—
	4	61	—	—
	30	67	—	—
	31	81	—	—
	74	273	199	1.9

(iv) However, further evidence was sought that cell compression played no part. Presumably if cell compression occurred it would act equally in both radial and tangential directions since each cortical cell is wholly surrounded by hyphae.

The average lengths of the radial and tangential walls in thirty-two cells picked at random from the fourth cortical row in each uninfected root were compared with those in the corresponding infected roots, see Table IV. No significant difference was found between the mean lengths of the radial walls, but the differences between the mean lengths of the tangential walls were found to be highly significant. It is evident therefore that little or no compression takes place in the tangential direction; it follows that it is unlikely to occur to any extent in the radial direction. The large differences in the length of the tangential walls must be due mainly to division of cells. This is further emphasized by the fact that the average length of the tangential walls in the four uninfected roots is almost twice that in the four infected roots; the ratio is  $39.0:20.5$ .

TABLE IV

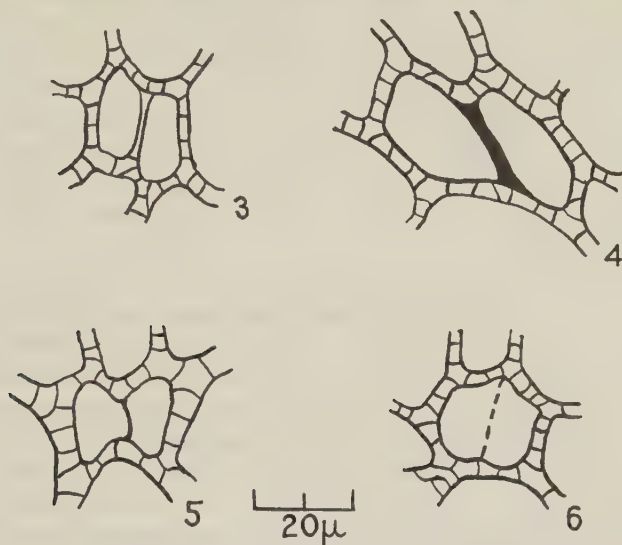
Mean Lengths of the Walls of 32 Cortical Cells in 4 Uninfected and 4 Infected Sublateral Roots of Larch

	Uninfected sublateral.	Infected sublateral.	Value of 't'.
	$\mu$	$\mu$	
Radial walls	$33.5 \pm 10.1$	$30.7 \pm 8.6$	1.66 (P = 0.1)
	$38.5 \pm 11.9$	$36.5 \pm 11.9$	0.95 (P = 0.4)
	$26.9 \pm 7.9$	$26.0 \pm 6.5$	0.68 (P = 0.5)
	$23.25 \pm 6.0$	$23.4 \pm 12.9$	0.05 (P = 0.9)
Tangential walls	$40.3 \pm 12.3$	$15.7 \pm 5.5$	14.35 (P = 0.01)
	$41.4 \pm 13.7$	$18.7 \pm 6.1$	12.22 (P = 0.01)
	$22.6 \pm 7.5$	$15.4 \pm 5.3$	6.25 (P = 0.01)
	$19.65 \pm 5.5$	$11.9 \pm 3.6$	8.92 (P = 0.01)

The four lines of inquiry set out above tend to confirm the hypothesis that the oblong cell shape in the transverse plane in sublateral roots infected by *B. elegans* is the result of the division of the cells. Nevertheless it must be noted that no undoubted example of cell division nor any dividing nuclei have been seen during the examination of many hundreds of sections. Text-figs. 3, 4, and 5 show the only cell structures which could possibly be interpreted as indicating the occurrence of cell division; a pair of cells was divided by a wall in which no hyphae were present, possibly due to the recent formation of the wall into which the fungus had not had time to penetrate. Text-fig. 6 shows a cell in which the protoplasm appears to be divided but no actual cell wall is visible; none of these structures, however, is conclusive. Furthermore, although division still occurs at 1,600  $\mu$  from the apex to judge by cell counts, the cortical cells cease to be meristematic at a distance of 300  $\mu$  from the apex; from this point onwards they are elongated and somewhat thick walled, so that it seems highly improbable that they can undergo cell division. However, the weight of the evidence so far available is in favour of cell division as the prime factor determining the oblong shape of the cortical cells in larch mycorrhizas. While this phenomenon has been investigated in detail in roots infected by *B. elegans*, it has also been observed on mycorrhizas formed by *P. involutus* and other unidentified mycelia where a balanced relationship has been established.

To sum up, for any particular root the number and shape of the cortical cells depends on the type of root and the type of infection. Thus an uninfected sublateral root will usually have from 30 to 40 elongated or isodiametric cells, a sublateral root infected by a black or yellow mycelium will be similar, but a sublateral root infected by *B. elegans* or other mycorrhiza-former will have 60 to 70 cortical cells, mostly oblong in shape. A lateral root, however, which has been attacked by *B. elegans* will have 80 or more cells, depending on its diameter; all the cells will be isodiametric in shape, since in lateral roots infection usually takes place well behind the apex, when the cells are definitely

too old to divide. According to Hatch and Doak (1933) long roots in pine are not infected because of their greater growth rate; this may be the case in larch since infection of lateral roots is usually only observed in material collected towards the end of the autumn season when, presumably, the rate of growth of the lateral roots has begun to slacken. Even then the tip is never infected,



TEXT-FIGS. 3-6. Cortical cells in T.S. of larch mycorrhizas infected by *B. elegans*.

probably because the hyphae cease growth before the rate of growth of the lateral roots has fallen to that of the sublateral roots. In view of this it is advisable when investigating a mycorrhizal association in larch to examine the branches of a monopodial system rather than the axis, for it is possible that the axis is not a sublateral root but an infected lateral root, in which case the features of mycorrhizal formation observed in it will not be typical of the particular fungal association as a whole.

Some authors have recorded hypertrophy of the cortical cells as one result of mycorrhizal infection in pine. In particular Hatch and Doak (1933) compared cortical cells in infected and uninfected short roots of pure culture seedlings and found that the cells were significantly larger in the infected roots. No such hypertrophy of the cells has been observed in larch roots by the present writer; rather the cells in infected roots tend to be smaller than in uninfected roots as a result of division. Further investigation of the cell reactions of both pine and larch is needed before final conclusions can be drawn, but there are indications that here we have two fundamentally different responses to mycorrhizal infection in the ectotrophic mycorrhizas of conifers. *Pinus* spp. with dichotomously branched mycorrhizas respond by enlargement of cells, *Larix* spp. with a monopodially branched system respond by division of



cells; it would be interesting to examine another monopodial form such as *Picea* in this connexion. If such reactions on the part of the root are an indication of a pathological condition, as has been suggested by Burges (1936), it is difficult to understand why the attacks by black fungi in larch do not result in any increase in cell division, even though many of these fungi are undoubtedly parasitic in action; similarly in pine it has been noted by Rayner (1934) that in general pseudo-mycorrhizas show none of the hypertrophy of the cells which is thought to be a constant feature of true mycorrhizas. It is therefore unsafe to conclude that cell reactions which are known in some cases to accompany pathological infection in plants are inevitably symptoms of disease. The reactions of the root as shown by the histology are not infallible guides to the total effect of the endophyte on the health and vigour of the host.

### *Intracellular infection.*

Only two types of intracellular infection have so far been observed in larch mycorrhizas. In the first, which is only found occasionally, the hyphae grow straight through the cells in a parasitic manner; these hyphae always belong to the 'yellow' or 'black' mycelia. In the second type, which is more frequent and will be referred to as the haustorial type, bladder-like swellings are observed attached to the cell walls and projecting into the cells; they are usually single but sometimes they are found in clumps. A similar condition has been noted in pine (Young, 1940). There is never any indication of the digestion of these hyphae such as occurs in endotrophic mycorrhizas. Infections by a number of different fungi have been found on occasion to exhibit this feature which would appear to be dependent for its development more on environmental factors than on the presence of any particular endophyte. Possibly it was intracellular infection of this kind which led Laing (1923, 1932) to describe some of the normal mycorrhizas of larch as semi-endotrophic. The use of such a term, however, is unfortunate since it implies the presence of features similar to those found in the true endotrophic mycorrhizas (Rayner, 1927). Actually there is only one feature common to both Laing's semi-endotrophic and true endotrophic mycorrhizas, viz. the invasion of cells by hyphae. Moreover, in view of the relative infrequency of this haustorial intracellular condition compared with that of the purely intercellular type, the conclusion that larch mycorrhizas are normally semi-endotrophic is certainly not justified.

Melin (1922) also held that intracellular infection was a normal feature of larch mycorrhizas and moreover that it was the primary condition, the intracellular net being the result of the 'squeezing out' of the mycelium from inside the cells as the resistance of the plant increased. Evidence from many young mycorrhizas examined during the present work does not support this view. Infection always begins by the formation of a thin mantle, which is followed by the development of a Hartig net from the outside inwards by the penetration of the hyphae into the middle lamellae; no intracellular infection has ever



been observed at this stage. Furthermore, Melin's conclusions were based on nursery material and abnormal features such as intracellular infection are often prevalent under these conditions; thus a totally erroneous impression of the importance of this type of attack would be gained.

#### *Cell inclusions.*

Frequently in the cortical cells one comes across shiny yellowish granules forming masses reminiscent of budding yeast cells. These masses are attached to the walls and may occur in both outer and inner cortical cells, in the endodermal passage cells, and in the tannin cells in the vascular bundle. They have been found in at least seven different infections, some of these infections being truly mycorrhizal, others showing pseudo-mycorrhizal features. The presence of the granules would appear to be correlated with infection inasmuch as they do not occur in uninfected roots. But their occurrence in many of the inner tissues of the root which are never penetrated by the mycelium precludes their being the direct result of the presence of the fungus in the cells; they cannot, for example, be the product of the digestion of intracellular hyphae.

An unsuccessful attempt was made to determine the composition of the granules. They are insoluble in alcohol, ether, or concentrated sulphuric acid, but soluble in Eau de Javelle and boiling 3 per cent. caustic potash. Osmic acid stains them dark brown, suggesting that they may be fatty or proteinaceous in character; however, all specific tests for oil or protein were negative. There is some evidence that these granules occur most frequently in moribund roots during the winter or early spring; but there is, as yet, no clue to their function or significance.

## VI. DISCUSSION

Any judgements concerning the significance of the observations which have been set out in this paper must of necessity be provisional. No final decisions can be reached until the various phenomena recorded have been studied in experimental culture under controlled conditions. This has not so far been possible. Nevertheless certain facts have emerged which call for some evaluation, even though at this stage it is possible to draw only tentative conclusions.

In both pine and larch the root systems show a differentiation into two morphological units, lateral and sublateral roots, alternatively named long and short roots; the differentiation apparently corresponds to the formation of long and short shoots in the stem. This development of two types of root is important for mycorrhiza formation, since each type behaves differently towards infection; the lateral roots normally remain uninfected, the sublateral roots rarely escape infection by one fungus or another. Reference has already been made to the suggestion of Hatch and Doak (1933) that the different behaviour of the two root types in pine is correlated with their different rates of growth, the fast-growing long roots remaining free from infection, the slower

growing short roots easily becoming attacked by certain soil fungi. There is some evidence that differential growth rates are similarly responsible in larch for the differences in the incidence of fungal infection in the lateral and sublateral roots. Although there is no explanation available as to why a fast growing root should be less easily attacked than a slow-growing one, it is clear that if confirmation of this is obtained it has great importance for the growth of the plant in various soils. For soil factors by influencing the rate of growth of lateral and sublateral roots might increase or decrease the number of mycorrhizas formed. Plants grown in a fertile soil in which the sublateral roots have a relatively high rate of growth might tend to have fewer mycorrhizas than plants grown in infertile soil where the rate of growth of even some of the laterals is too low for the roots to remain uninfected. Hatch (1937) did in fact find that fewer mycorrhizas were formed in fertile than in infertile soil; he attributed this to the high internal salt concentration of the plants which had been grown on a rich substrate, but it may well be that a high rate of growth in the sublateral roots is also a factor.

Throughout this paper it has been assumed that a knowledge of the range of structural features found in the mycorrhizas of larch might enable one to determine by the criterion of structure alone the value of any given infection to the plant. It is realized, however, that a knowledge of mycorrhiza formation in native habitats together with the experimental testing of the effect of various endophytes on the health and vigour of the plant is necessary before the value of a mycorrhizal association can be estimated solely on grounds of structure. In the course of this investigation it became evident that there is at least one structural feature which can be used to distinguish between the balanced association of a mycorrhiza and the unbalanced association of a pseudo-mycorrhiza. Such a feature is supplied by the presence in mycorrhizas and the absence in pseudo-mycorrhizas of oblong-shaped cells in the outer cortex, due to the division of the cells by radial walls. When the presence of oblong cells in the cortex of infected larch roots is used to classify an association as a balanced one, it is found that the other structural features which accompany this phenomenon are similar to those which occur in pine mycorrhizas known from experimental cultures to be balanced; furthermore, the deviations from this condition sometimes found in infected larch roots are of the same type as the deviations in structure observed in pine roots attacked by deleterious fungi. It is on the assumption that a fundamental similarity exists between the mycorrhizal relations of the two genera that the value of each of the seven types of infected root described in section IV has been estimated.

Accordingly, until there is evidence to the contrary a mycorrhizal association in larch may be said to be 'balanced' when its structure is as follows. The mantle, which varies from 10 to 60  $\mu$  in width according to the endophyte, consists of hyphae closely woven to form a definite structure depending on the particular fungus concerned; the mantle encloses two or three layers of dead cortical filaments, which are adjacent to a single tannin layer containing

mainly squarish tannin cells with a few granular cells. The Hartig net extends across the outer cortex to the inner cortex and the cell walls are not swollen and contain only one strand of hyphae; the outer cortical cells are oblong in shape and at least 60 in number; intracellular infection of any kind is absent. The greater majority of the deviations from this structure are clearly due to the encroachment of the fungus at the expense of the root, viz. very wide fungal mantles containing isolated cortical cells, very swollen cortical walls containing two or more strands of hyphae, intracellular infection. Occasionally the reverse condition is found in which the fungus apparently finds difficulty in establishing itself as shown by the almost complete absence of mantle and Hartig net.

In theory, under natural conditions a sublateral root may develop in four possible ways. Firstly, it may remain uninfected, an infrequent occurrence; in this case it remains unbranched. Secondly, it may become infected by *B. elegans*, or many other mycelia so far unidentified, and form a balanced association. Thirdly, it may become infected with one of these mycorrhizal fungi but form an unbalanced association; possibly types 2 and 3 are produced in this way. Finally, it may be attacked by certain 'black' fungi which tend to form only pseudo-mycorrhizal associations. In practice, unless the identity of the endophyte is known, it is impossible to distinguish between the third and fourth of these possibilities on grounds of structure alone, since an unbalanced association is produced in each case. It may be found that an unbalanced association with a potential mycorrhiza-former will nevertheless show division of the outer cortical cells, in which case it should be possible to distinguish this condition from the true pseudo-mycorrhizal state where no such division has been found to occur. Before this can be done with certainty, however, considerably more experience will be required of the possible variations in the structure of larch mycorrhizas.

Which of these four possibilities is realized in any particular root will depend on what species of fungi are actually present in the soil immediately surrounding the root and on internal factors in the root itself; the latter are as yet unknown, apart from the two already mentioned, viz. high internal salt concentration and rate of root growth. Both the external factors such as the fungi and the internal factors will be almost wholly determined by soil conditions. Edaphic factors will largely determine on the one hand, the species and vigour of the fungi available for mycorrhiza formation, and on the other the growth rate of the roots and their power of resistance to infection. The examination of all the roots of naturally regenerated seedlings reveals that frequently the mycorrhizas of any one seedling have been formed by a number of different fungi; it therefore appears probable that many habitats are a mosaic of small areas in each of which the soil conditions vary more or less widely from the others. The general result of mycorrhiza formation for any individual will then depend on the proportion of beneficial and deleterious associations present in its roots. Thus the discovery of pseudo-mycorrhizas in a plantation



is only of significance if they occur frequently. Nor does the presence of hyphae of a known pseudo-mycorrhiza-former in the soil necessarily imply that pseudo-mycorrhizas will be formed. Hyphae of 'atrovirens' type have been observed in many soils in which no pseudo-mycorrhizas could be found. It follows that in appraising the part played by the various soil factors in mycorrhiza-formation more weight should be placed on those that affect the health and vigour of the roots than on those which affect the presence or absence of the fungus. Hatch (1936) considered that the prime necessity in the establishment of exotic conifers is the inoculation of the soil by suitable mycorrhiza-formers; this is undoubtedly the case in many countries. But in the British Isles, where many potential mycorrhiza-formers with larch exist in most soils, attention should be directed more to the study of edaphic factors and their bearing on the formation of balanced associations in larch than to the possibilities of adding known mycorrhiza-formers to the soil.

## VII. SUMMARY

1. The roots of European larch can be grouped into two classes, lateral, and sublateral roots, corresponding to the long and short roots of pine. The majority of the mycorrhizas are normally formed by the infection of the sublateral roots, a few by the infection of lateral roots.

2. *Boletus elegans* and *Paxillus involutus* have been isolated from larch mycorrhizas. *Mycelium radialis atrovirens* from pseudo-mycorrhizas.

3. Evidence is produced that infection of the sublateral roots by a mycorrhiza-former such as *B. elegans* results in an increase in the number of cortical cells in the root as compared with either uninfected sublateral roots or pseudo-mycorrhizas.

4. Detailed descriptions are given of the structural features resulting from infection by *Boletus elegans*, *B. viscidus*, *Paxillus involutus*, a yellow hymenomycete mycelium, *Mycelium radialis atrovirens* and two unidentified mycelia.

5. The general features of mycorrhiza formation in larch are discussed.

I wish to express my grateful thanks to Professor W. Neilson-Jones, M.A., and Dr. M. C. Rayner for criticism and advice in the preparation of this paper.

## APPENDIX

### *Mycorrhiza Formation in Experimental Culture*

Reference has been made in the present paper to the necessity of studying the various mycorrhizal associations of larch in experimental culture if an accurate estimate is to be gained of the effect of any particular association on the health and vigour of the plant. An essential preliminary for such work is a technique for inducing larch seedlings to form mycorrhizas with the particular fungus to be investigated.

With this object in view a series of pots containing soil from a larch stand were sown with European larch seeds and inoculated some  $2\frac{1}{2}$  months later



with living mycelium of either *B. elegans* or *P. involutus*. The inocula were derived from glucose agar cultures which were chopped up and mixed with the soil round each seedling. At the end of the first growing season examination of the roots showed that this method of inoculation had been ineffective since no mycorrhizal associations had been formed with either of the fungi. Nevertheless, the seedlings had formed some unbalanced associations with mycelia already present in the soil. The failure of the experimental fungi to establish themselves was thought to be due to three causes, the unsuitability of the soil, the type of medium on which the cultures were growing at the time of inoculation, and the method of inoculation; it seemed probable that the last two factors played a larger part in the inhibition of growth than did the soil factor. Thus the use of agar cultures was probably unwise since these may easily become exhausted of nutrients before the slow-growing mycelia have had time to establish themselves in the soil; in addition no attempt had been made to place the inocula in contact with the young roots, and the lack of such contact possibly contributed to the negative result.

Accordingly the following year cultures were set up in which particular attention was paid to the types of substrate used for inocula and the methods of inoculation. Young (1936) had found glass-sided boxes of great assistance in observing the formation of mycorrhizas in pine and a modified form of his containers were adopted in this and subsequent experiments with larch seedlings. This made it possible to observe directly the behaviour of inocula in the soil and to place them in direct contact with the roots by removing the glass panels.

The boxes were constructed of  $\frac{1}{2}$  in. teak with two sloping panels of glass which could be moved up and down in grooves; the panels were protected by zinc sheets painted black. The internal measurements were  $11\frac{1}{2} \times 5\frac{1}{2}$  in. at the bottom,  $11\frac{1}{2} \times 8$  in. at the top, and 12 in. deep; the base was raised 1 in. above the ground and was perforated at intervals for drainage. The boxes were filled with the artificial soil mixture given below; its pH was 5.4:

Meadow loam	.	.	.	.	50 per cent. by vol.
Sand	.	.	.	.	$12\frac{1}{2}$ „ „
Sandstone chips	.	.	.	.	$12\frac{1}{2}$ „ „
Sawdust Compost C 6a (Rayner 1936)					25 „ „

European larch seeds were sterilized in 0.1 per cent. mercuric chloride before sowing. In each box a dozen seeds were evenly spaced in a row about  $\frac{1}{4}$  in. from each of the glass panels to ensure that the radicles rapidly came in contact with the glass. The following types of culture were used for inoculation, the inocula being placed against the root-tips in all cases except when inoculation was carried out at the time of sowing, in that case the inocula were placed beside the seeds.

A—Beerwort agar cultures of *B. elegans* or *P. involutus* at the time of sowing.

- B—Steamed soil cultures of *B. elegans* or *P. involutus* 6 weeks after sowing.  
C—Wheat or oat seed cultures of *B. elegans* or *P. involutus* 10 weeks after sowing.  
D—Wheat or oat seed cultures of *B. elegans* or *P. involutus* 10 weeks after sowing.  
E—Sawdust compost cultures of *B. elegans* 10 weeks after sowing.  
F—Portions of hymenium from ripe sporophore of *B. elegans* 6 weeks after sowing.

The growth of the seedlings under all treatments was good. Examination of the root systems at the end of the first season showed that none of the inoculation methods had been successful with either *B. elegans* and *P. involutus*, although the formation of incipient mycorrhizas with mycelia already present in the soil indicated that the seedlings were capable of forming associations. It is therefore clear that neither a reservoir of nutrients, such as was supplied by the seed cultures, nor contact with living roots provided the necessary conditions for mycorrhiza formation with the experimental fungi. Before finally deciding that this failure was due to soil conditions inimical to the fungi and not to methods of inoculation a third method was tried, based on the fact that larch seedlings can easily become infected by *B. elegans* under pure culture conditions where the balance is easily weighted in favour of the fungus (cf. Melin's experiments, 1922). It was thought that seedlings infected in this way would remain infected after transference to soil.

The seedlings were raised in glass containers ( $1\frac{1}{2}$  in. diam.  $\times$  4 in.) which had been filled to a depth of  $1\frac{1}{2}$  in. with one or other of the following materials. (1) Osmunda fibre. (2) Sorbex 7 parts, charcoal 1 part, sand 1 part. These materials were well moistened with the following nutrient solution: glucose, 0.5 gm.;  $\text{KH}_2\text{PO}_4$ , 0.2 gm.;  $\text{NH}_4\text{Cl}$ , 0.1 gm.;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.01 gm.; distilled water, 100 cc. The Osmunda fibre cultures were sterilized by autoclaving at 15 lb. pressure for 30 minutes, the Sorbex cultures by steaming for 30 minutes on three successive days.

After centrifuging for 5 minutes in distilled water, the seeds were sterilized in calcium hypochlorite (Wilson, 1915) and put to germinate on Melin's glucose agar so that any contamination could be easily detected. When the testas had split the seeds were transferred singly under aseptic conditions to the containers which had been inoculated six days previously with *B. elegans*. Controls were set up without fungal inoculation. The seedlings developed well and were very vigorous; the presence of the fungus in no way impaired their vitality. About ten days after planting in the containers the whole contents of the latter were transferred to the boxes so that the roots were undisturbed. No true estimate of the mortality rate of the seedlings can be given since a number of the transplants were accidentally damaged by birds. However, the majority of the undamaged seedlings raised in the Sorbex cultures appeared to thrive, but there was a high death-rate with the Osmunda fibre cultures; this was due, no doubt, to the open nature of the substrate

leading to drying out of the roots where these did not come into intimate contact with the soil. Examination of the inoculated transplants at the time of transference showed that no parasitism had occurred although the fungal hyphae had wound themselves thickly round the hypocotyl. The outer cortical cells had been pushed aside by the fungus in some cases but there was no entry into the cells and the appearances seemed to be due purely to mechanical pressure.

The composition of the soil mixture in the boxes was as follows:

Meadow loam . . . . .	20 per cent. by vol.
Sand + sandstone chips . . . . .	30 " "
Charcoal . . . . .	10 " "
Hop waste compost C5 (Rayner, 1936) . . . . .	40 " "

The components were steam sterilized before mixing; the pH value of the final product was 6.0–6.2. The following series of boxes were set up in duplicate:

- A—Seeds placed directly on pads of sterilized Sorbex in the boxes.
- B—Seedlings raised in containers of Sorbex before transference.
- C—Seeds placed directly in the boxes on pads of Sorbex previously infected with *B. elegans*.
- D—Seedlings raised before transference in containers of Sorbex previously infected with *B. elegans*.
- E—Seeds placed directly in the boxes on pads of Osmunda fibre previously infected with *B. elegans*.
- F—Seedlings raised before transference in containers of Osmunda fibre previously infected with *B. elegans*.

The growth of the seedlings was outstandingly good. After five months the roots were examined; it was found that no mycorrhiza formation had occurred and that the mycelium of *B. elegans* had apparently died out. A repetition of this experiment in a sterilized heath soil met with no better success even though extra pads of Sorbex or Osmunda fibre were placed below the radicles at the time of transplanting so that the roots would grow down into the mycelium.

While no explanation of these repeated failures to procure mycorrhizal associations with *B. elegans* or *P. involutus* can be offered, it is clear that the solution to the problem is to be sought in the soil conditions. However, when the experiments are examined with a view to discovering a possible inhibiting factor in the soils concerned, it is evident that no single factor such as poor soil aeration, high pH value, or low humus content was operating in these cultures. It must therefore be concluded from experimental culture that there are some soil conditions as yet unknown, which need to be satisfied if mycorrhiza-formation with *B. elegans* or *P. involutus* is to be established with larch seedlings.



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EXPLANATION OF PLATE II

Illustrating the article by Dr. Eastoe How on 'The Mycorrhizal Relations of Larch. III. Mycorrhiza Formation in Nature'.

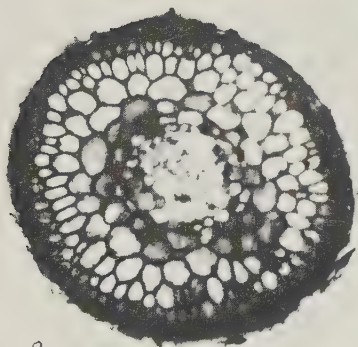
Fig. 1. Mycorrhizas of European larch, showing monopodial branching; endophyte unknown. ( $\times 2$ .)

Figs. 2-5. Transverse sections of larch roots showing various types of infection. ( $\times 87$ .)  
Fig. 2. Type 1; endophyte *Boletus elegans*. Fig. 3. Type 3; endophyte *Paxillus involutus*.  
Fig. 4. Type 5; endophyte yellow hymenomycete mycelium. Fig. 5. Type 6; endophyte *Mycelium radialis atrovirens*.

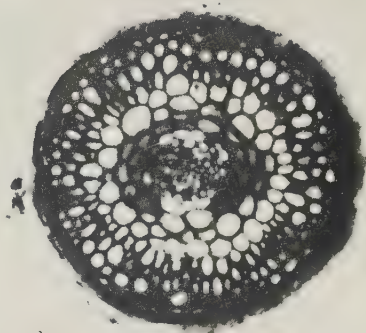




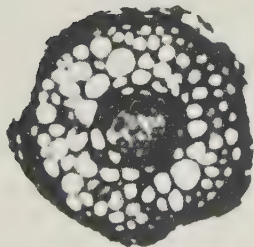
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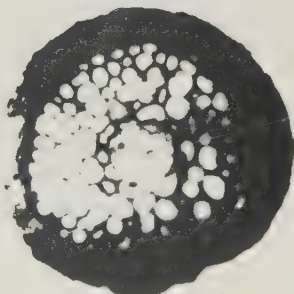
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HOW — LARCH MYCORRHIZA.





# Parasitism in the Santalaceae

BY

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With Plate III and twenty-one Figures in the Text

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## I. INTRODUCTION

THE Santalaceae, including Grubbiaceae and Myzodendraceae, comprise 26 genera with 250 species (Willis, 1931). Certain genera are known to be semi-parasites, but the majority have not yet been investigated from this point of view; apart from *Thesium* and *Santalum* the information is scanty. The parasitism of Santalaceae was first demonstrated by Mitten (1847) in *Thesium*. 'The remarkable nature of the root of *T. linophyllum* has apparently hitherto altogether escaped attention. Indeed, from the general appearance of the plant, there is nothing to excite suspicion; nor will there be any trace left of its parasitical attachment to the roots of the surrounding plants.' This remark of Mitten applies equally well to other semi-parasites among Santalaceae. Planchon (1858) first observed the parasitism of *Osyris alba*. Scott (1874), finding it difficult to move *Santalum album* without a large ball of earth, arrived at the idea, already suggested by Decaisne's (1874) experiments on Rhinanthaceae, that its roots were attached to those of other plants. Shirai (1877) discovered the parasitism of *Buckleya quadrila* in Japan.

Among the earlier accounts of the structure of the haustoria in Santalaceae, those of Pitra (1861), Solms-Laubach (1867-8), Kusano (1902), and Barber (1907) are the most important. Pitra studied the structure of the haustorium in *Thesium ramosum* and stimulated investigations in this direction. Solms-Laubach in *T. pratense* confirmed the findings of Pitra as regards the host-parasite relation. He also made a superficial study of the haustoria of *Osyris alba* and *Santalum album*. Kusano, examining the haustorium of *Buckleya quadrila*, explained the structure of the nucleus and the presence of cambium

in it. A detailed, though not exhaustive, investigation of the parasitism of *Santalum album* was undertaken by Barber, who placed great emphasis upon the morphology and development of the haustoria; he did not, however, examine the early development of the latter. Between 1907 and 1940 several short papers dealing with certain aspects of the parasitism of other members of Santalaceae have been published, viz. Herbert (1920) on *Fusanus spicatus*, Exocarpus, and Choretrum, Hedgecock (1915) on *Comandra umbellata* and *C. pallida*, Woodcock (1920) and Moss (1926) on *C. pallida*. In this investigation an attempt has been made to study the structure of the haustorium in *Santalum album*, *S. lanceolatum*, *Osyris arborea*, *Thesium Wightianum*, *Scleropyrum Wallichianum*, *Exocarpus aphylla*, and *Choretrum glomeratum*. Certain details that have escaped the attention of previous investigators have been discovered. The parasitic nature of *Scleropyrum* had not yet been established.

## II. MATERIAL AND TECHNIQUE

*Santalum album* has a wide range of hosts. Barber (1907) records 160 and Rama Rao (1918) about 40 species of angiosperms as being attacked by the sandal tree, the majority of the 160 species occurring in the sandal zone of Mysore. Material of haustoria was collected in the field from a diversity of hosts and also from cultures on definite hosts, which proved very useful in providing the younger stages. The haustoria of *Osyris* were collected in the field from several hosts, including grasses and *Botrychium virginianum*, which has not hitherto been reported as being the host of a semi-parasite. The haustoria on the soft roots of *Botrychium* are specially well suited for sectioning and provided good material for a thorough study of their structure. *Thesium* haustoria were collected from a number of weeds, including grasses. Since the anatomy of the *Thesium* haustorium has been investigated in many species, attention was mainly confined to one on a grass-root which exhibited certain peculiarities. Haustoria of *Scleropyrum* were obtained from the roots of several trees and shrubs, but the young stages could not be found in nature. The seedlings take a long time to establish themselves, so that the early development of the haustorium could not be investigated.

Younger stages were fixed for twenty-four hours in medium chromacetic acid and older ones in formalin alcohol and acetic acid. The former were washed after fixation, dehydrated, and embedded in paraffin. The latter were usually hard and several methods of softening them were tried. For moderately hard material acetic acid treatment and the cellulose acetate method of Williamson (1921) were found suitable. For harder material hydrofluoric acid was used for softening and demineralizing, followed by embedding in celloidin, as suggested by Jeffrey (1928) and Wetmore (1932). Fairly thin sections can be cut, if the infiltration of celloidin is perfect. The sections were cut with a Reichert sliding microtome and were stained mainly with Ehrlich's haematoxylin, safranin and light green, safranin and aniline

blue, and gentian violet. Paraffin sections were stained with iron-alum-haematoxylin in addition to the above combinations. Diverse tissues of the haustorium were macerated and examined in glycerine jelly slightly coloured with safranin.

### III. STRUCTURE OF THE HAUSTORIUM

#### (i) *The simple haustorium.*

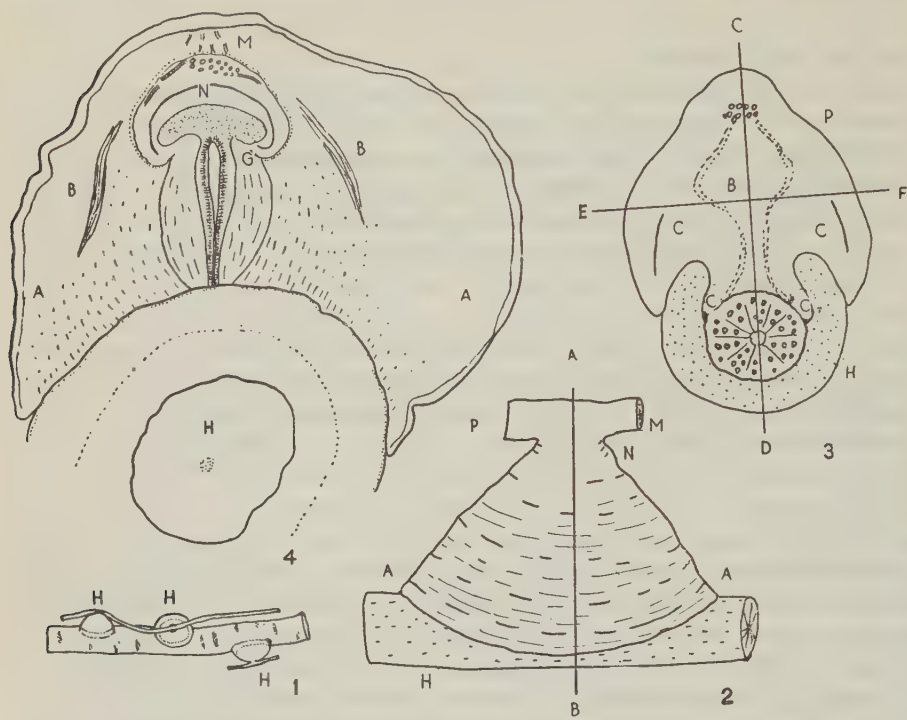
(a) *Santalum album* and *S. lanceolatum*. A typical mature haustorium of Santalaceae is roughly conical (Text-figs. 1 and 2). The apex of the cone at which the haustorium arises from the parent root is called the neck (Text-fig. 2, N), while the broad base which is in contact with the host root is called the apex (Text-fig. 2, AA); the intervening part constitutes the body of the haustorium. The neck remains more or less unchanged throughout the life of the haustorium, while the apex and the body undergo many changes. In its final condition the body consists of a central nucleus and the peripheral cortex, while the apex can be similarly divided into the central sucker and the peripheral cortex which is a continuation of that of the body. The vascular bundles pass from the parent root through the neck and traverse the periphery of the nucleus, finally extending through the sucker to the host root (Text-fig. 3).

The haustoria of *S. album* arise laterally on the roots, but are not endogenous. Not a single terminal haustorium was found. The young haustorium is formed by the epidermis and cortex of the root; later, when the vascular system develops, it becomes joined to that of the parent root. Sufficient data are not at present available as to the causes inducing the formation of haustoria, although Heinricher (1926) attributes it to a chemical stimulus proceeding from the host root; both contact and chemical stimulation appear to play an important part. The young haustoria are easily distinguished from the endogenous lateral roots and appear as small hemispherical outgrowths. As lengthening proceeds, the free end comes in contact with the host and gradually flattens so as to accommodate itself to the contour of the root of the latter. At this stage the young haustorium already consists of a narrow neck, a cushion-shaped body, and a broad apex. The body consists of massive parenchymatous tissue, the centre of which is occupied by the nucleus, whose cells are meristematic and rich in cytoplasm. The apex of the haustorium plays an active part in the penetration of the cortex of the host.

The further development of the haustorium varies according to the nature of the host. If the latter is soft and delicate (Pl. III, Fig. 1 and Text-fig. 9), the apex applies itself firmly to the host and the cells of the outermost layer elongate radially and acquire rich cytoplasm with large nuclei, i.e. they become glandular. The secretion of these glandular cells appears to have a solvent action on the cell walls of the host, with the result that the outer cells of the latter disintegrate and make way for the growing sucker which is a continuation of the nucleus. Simultaneously there is rapid growth of the



parenchymatous peripheral part of the body of the haustorium, so that it extends round the glandular area of the sucker and comes in contact with the host. This spreading tissue, which in sections generally appears as a layer of varying thickness on either side of the host root, constitutes the 'clasp-



TEXT-FIGS. 1-4. Fig. 1. Haustoria of *Scleropyrum* showing habit. Fig. 2. Diagrammatic sketch of enlarged haustorium. P, parasite; M, mother root; N, neck and AA, apex of the haustorium in contact with H, host root. Fig. 3. Longitudinal section of the haustorium along the plane AB shown in 2. P, parasite; H, host; CC, cortex of the body and the sucker; B, nucleus; EF, plane of transverse section, and CD that of longitudinal section along the long axis of the haustorium. Fig. 4. Haustorium of *Santalum* on *Dodonaea* showing the internal gland. H, host root; A, cortical folds; B, collapsed layers; M, mother root vascular strand; N, nucleus; G, gland. Dotted lines show the direction in which the cells are disposed. ( $\times 35$ .)

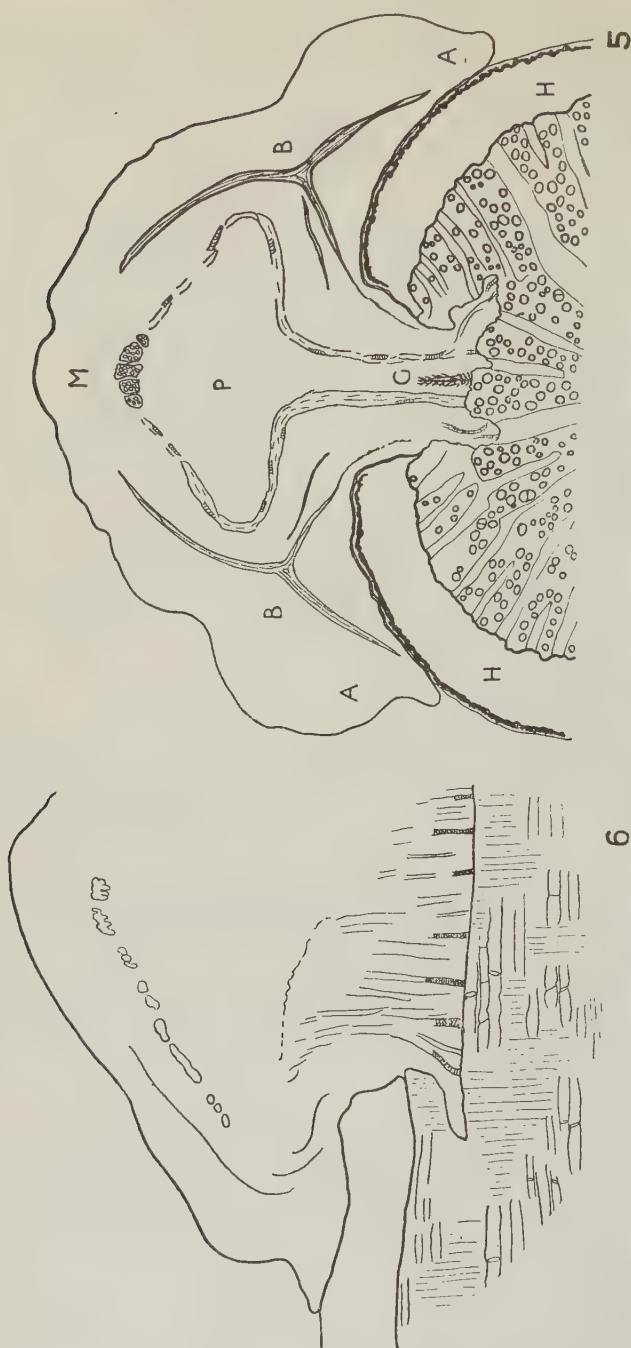
folds' (Text-fig. 9 AA). The surface of the folds, that comes in contact with the host, still possesses the capacity to form glandular cells which play a part in the digestion of tissues, and such secondary glandular activity is sometimes recognizable. As the massive body of the haustorium enlarges, its peripheral parts seem to grow less rapidly while the central meristematic nucleus remains active. Owing to this disparity in the rate of growth, the two regions come to be separated by a layer of crushed cells, which runs more or less parallel to the general trend of the cells of the nucleus (Text-fig. 9 BB). These layers first appear midway between the apex and the neck and gradually extend in either



direction (Text-fig. 4 BB). They represent the 'Trennungsstreifen' of Solms-Laubach, the 'collapsed layer' of Barber, and the 'separation strip' of Moss.

Smaller vascular strands appear to branch off from the cylinder of the parent root into the haustorium, entering the parenchymatous tissue through the neck. The vascular elements are actually differentiated from the meristematic tissue of the haustorium around the nucleus, a process which commences at the neck and proceeds to the apex. Within the neck the vascular strands spread out as a number of smaller strands, which traverse the body at the periphery of the meristematic nucleus. Sometimes the diverging vascular strands run at a deeper level and parallel to one another, until they reach the vascular strands of the host, which by now are exposed, partly through the action of the glandular cells and partly through the ingrowth of the 'sucker' formed by the nucleus. Thus, the vascular cylinder of the haustorium resembles an inverted flask, the mouth of which is applied to the vascular system of the host, while its body, which is near the neck of the haustorium, is usually spherical but sometimes longitudinally compressed, in various ways. As the vascular system of the host is approached, the phloem of the haustorium appears to lag behind while the xylem extends rapidly, its elements become attached to the xylem-elements of the host. After the penetration of the sucker into the cortex of the host the undigested layers of the latter are to be found between the sucker and the clasping folds and are separated from the central cylinder of the host root.

If a hard and woody root has to be penetrated (Pl. III, Fig. 2, Text-figs. 4 and 5) the greater resistance induces certain changes in the growth of the haustorium. The glandular cells formed by the young haustorium in such instances fail to effect penetration. Consequently, there is formed an internal gland which extends deep into the nucleus and has a slit-like opening at the apex of the haustorium (Pl. III, Fig. 2, and Text-fig. 4). The gland is schizogenous in origin and is made up of two parts, a narrow duct and a wide cavity, the surface of both being lined by a layer of palisade cells, rich in protoplasm. This glandular epithelium appears to be mainly responsible for the production of the secretion required for dissolving the host tissue. The cells, immediately around the gland, also possess rich protoplasm and by their staining reactions seem to be closely connected with the activity of the gland. The latter is well supplied with vascular connexions from the main vascular strands (Text-fig. 4, N, G, and M). After this, additional peripheral growth leads to the formation of clasping folds, grasping the host firmly, while the digestion of the host cortex is effected by the secretion of the gland. Thus the sucker is able rapidly to grow into the cortex. Further growth within the body of the haustorium leads to the obliteration of the gland. In some cases disorganized remnants of it can be seen even in a mature haustorium (Text-fig. 5 G). As the latter penetrates into the host its tissue, especially that of the sucker, spreads outwards from the axial line, so that the cortex of the host is raised all round and becomes separated from the wood along the cambium.



TEXT-FIGS. 5 and 6. Fig. 5. Longitudinal section of the haustorium of *Scleropyrum* on *Schleichera trijuga*. A, cortical folds; B, collapsed layers; M, vascular strand from the mother root; P, pith in the loop of the vascular cylinder; G, vestiges of the gland; H, host root. The growth of the sucker along the cambium of the host can be recognized ( $\times 28$ ). Fig. 6. Median longitudinal section of the haustorium of *Scleropyrum*, along the long axis, showing the course of the vascular strands and their attachment to the vascular elements of the host. ( $\times 28$ .)

Wherever the sucker comes in contact with the xylem of the host the cambium of the latter is invariably destroyed; but in other places it persists, adhering to the raised cortex and later on producing a small amount of secondary tissues.

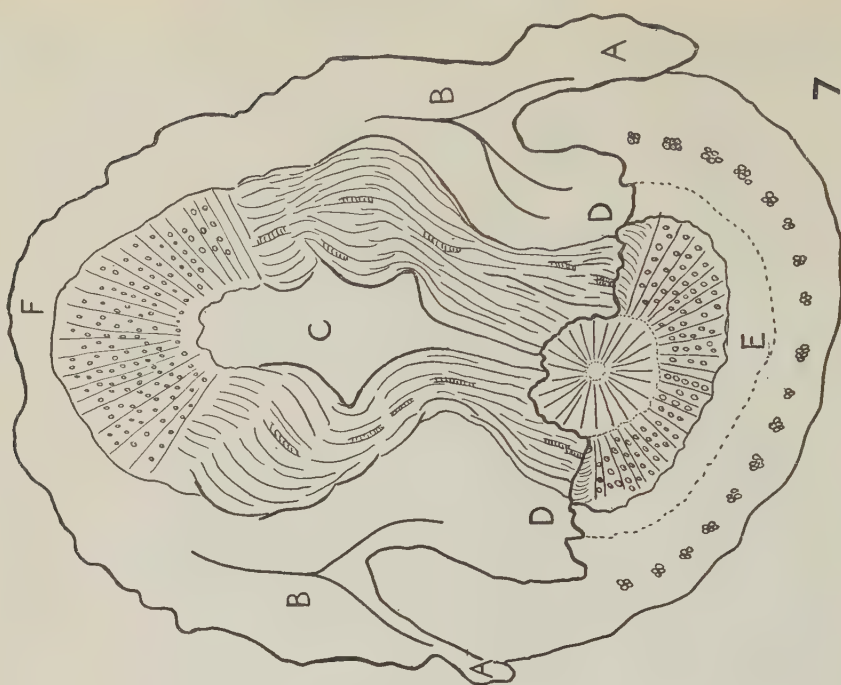
When the sucker reaches the woody cylinder of the host the rate of growth of its central portion decreases, while the marginal part continues to enlarge, and thus a large area of the wood is brought under its control. The cells of the sucker that come in contact with the wood of the host are usually elongated, thin-walled with rich cytoplasm and large nuclei, and appear to be glandular in function. This feature is especially noticeable along the invading margin of the sucker, where the products of glandular activity can be seen as brown masses, sometimes lodged deeply within the host tissue. The penetration of the sucker into the host is effected, as seen above, both by mechanical and chemical means.

In the meantime the vascular connexion between the sucker and the body of the haustorium is established. Around the region which was previously occupied by the gland, rows of vascular elements, mainly xylem, are differentiated. They form a continuous hollow cylinder, extending from the neck to the sucker. At its forward end, just before meeting the wood of the host, the vascular cylinder spreads out and becomes divided into individual strands separated by glandular cells. In this way each xylem element can come in contact with a xylem element of the host. The frayed nature of the vascular cylinder of the sucker at its forward end is due to the fact that only those cells of the glandular layer that have penetrated deeply and have come in contact with one or more of the xylem elements of the host are transformed into xylem elements. They form the terminations of a long chain of vascular elements which are concerned in the passage of liquid from the host to the parasite (Text-figs. 5, 6, and 7).

A definite cambium lies external to the xylem-cylinder of the sucker and is continuous with that of the parent root. Sometimes it leads to secondary growth in the haustorium. Phloem elements of the ordinary angiospermous type are not present; elongated cells with rich protoplasmic contents, however, form a definite zone external to the cambium which is more or less continuous from the neck of the haustorium to some distance into the sucker.

According to Barber (1907) slightly above the widest part of the vascular loop there is an interrupted zone in which the vascular elements show disintegration and a yellow colour develops owing to changes in the vessels. Investigation of the haustoria of *Santalum album*, *S. lanceolatum*, *Scleropyrum* *Osyris*, and *Thesium* failed to disclose such an interrupted zone. In the position mentioned by Barber the individual strands sometimes diverge so that the cylinder appears swollen when seen in longitudinal section (Pl. IV, Figs. 3, 6, Text-figs. 5, 6, 7, and 8). In a traverse section at this level the diverging strands are cut obliquely, and it is perhaps this appearance that gave Barber the impression of an interrupted zone (Text-figs. 14-21).

In macerated preparations, mounted in glycerine jelly with safranin, the





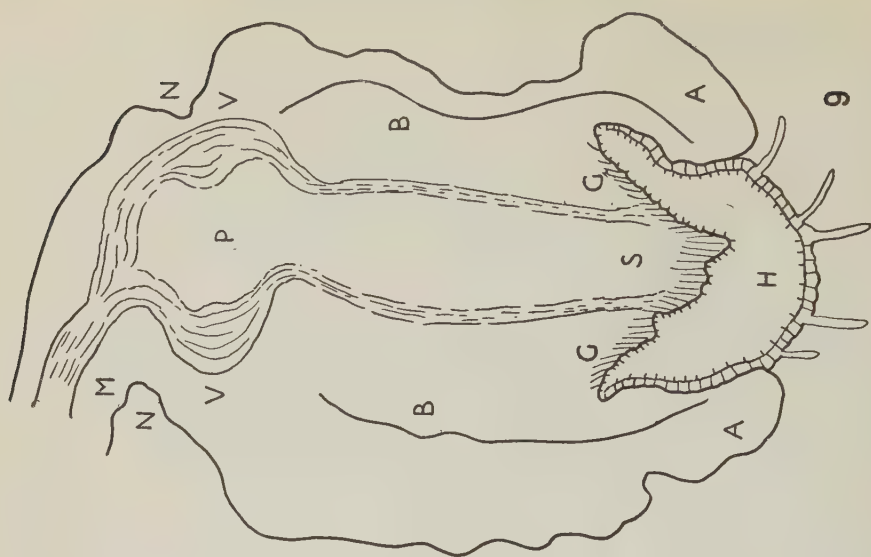
xylem elements of the haustorium between the neck and the apex are of the nature of vessels; no tracheides were found, but the segments of the vessels are short and sometimes distorted so as to appear like tracheides. The perforations are situated on the end walls or sometimes on the side walls. The vessels contain no protoplasm or carbohydrates as described by Benson (1910) in *Exocarpus* and *Thesium*. When they reach the xylem elements of the host, contact is effected by apposition of the pit-apertures of the members concerned and not by penetration of the host elements, as described in *Comandra* (Moss, 1926).

When once firmly established on a suitable host the haustorium of *Santalum album* develops secondary tissues. This was observed in an haustorium on *Jasminum* (Text-fig. 7), where a considerable amount of secondary xylem was formed connecting that of parent and host roots. Barber (1907) sometimes found difficulty in distinguishing between the secondary tissues of haustorium and host, but this was not so in the instance studied by me, where the attachments of the xylem elements of the parasite to those of the host were clearly seen (Text-fig. 7). As the haustorium increases in girth as a result of secondary thickening, the new xylem elements become attached to the newly formed xylem elements of the host, and this seems to continue as long as the two cambia remain functional, especially near their point of junction (Text-fig. 7, DD). As the haustorium becomes permanent, the nucleus becomes parenchymatous, the pith thus formed being continuous with that of the parent root.

A similar development of secondary xylem has been observed in an haustorium of *S. album* attached to the roots of the same species, an instance of self-parasitism. The early development of the haustorium was normal, except that the invading sucker did not completely destroy the cambium of the host, but detached it along with the cortex. This cambium remained active and formed secondary tissues (Text-fig. 8). While secondary tissues were developing in the haustorium, its parenchymatous body gradually shrivelled and became reduced to a thick hard scaly structure, adhering to the host (Text-fig. 8, ABA). As secondary growth of the host root proceeded, the sucker became embedded in its wood. It appears to resist this encroachment by the activity of its gland cells. As the secondary wood of the host increased, the embedded sucker grew at the same rate and also produced secondary xylem

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TEXT-FIGS. 7 and 8. Fig. 7. Haustorium of *Santalum* on *Jasminum* sp.? A, cortical folds; B, collapsed layers; C, pith inside the vascular cylinder; F, haustorium; D, the line of demarcation between the host and parasite tissues. Note the secondary wood and its connexion with that of the host E, at points DD. ( $\times 28$ .) Fig. 8. Longitudinal section of an old haustorium of *Santalum*. This is the only case yet recorded of a permanent attachment of an haustorium to the host with every provision for continued growth of both. A, cortical folds, now flat, due to the growth of the host root; B, haustorium very much reduced; C, cortex of the host; W, wood of the host showing growth rings (1-5); P, pith in the middle of the vascular cylinder. At x new xylem elements from the sucker are diverted to establish contact with the new woody elements of the host. ( $\times 17$ .)

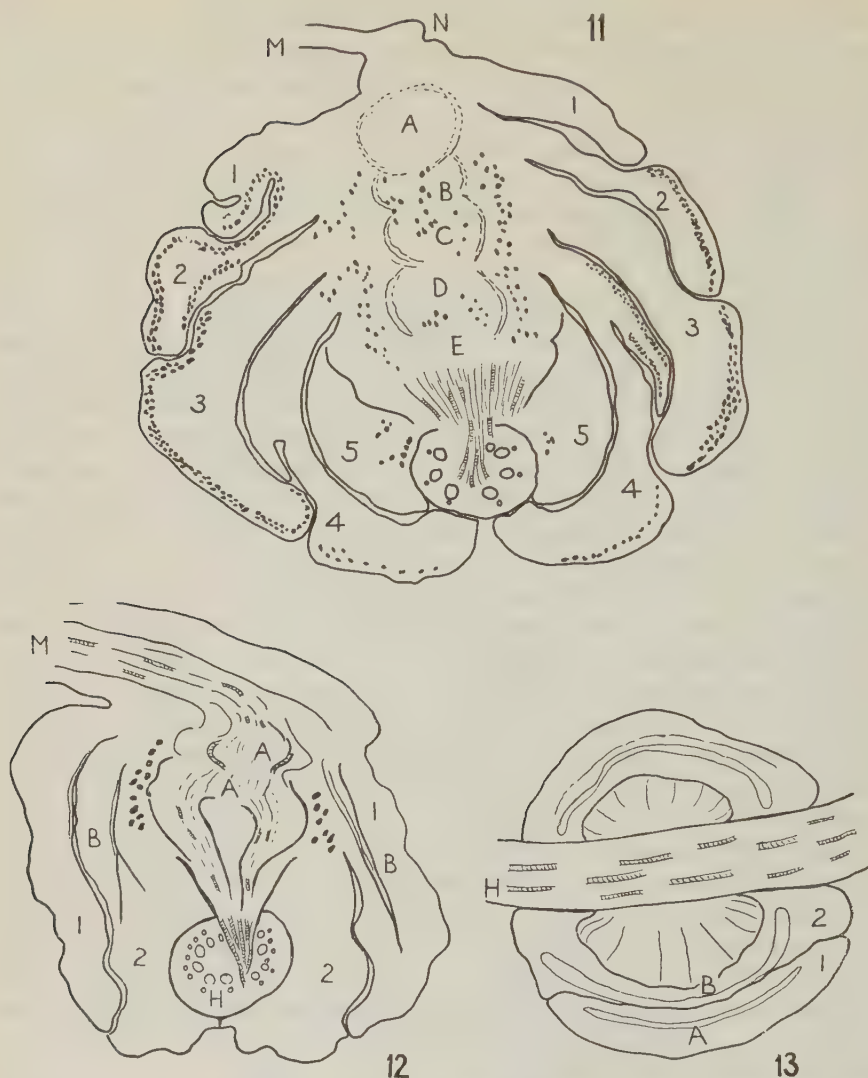


elements which became attached to the newly formed xylem elements of the host. In one example five growth-rings were present in the wood of the host, and the sucker was attached to the first formed one over its whole surface; where it extended through the rings subsequently formed it produced secondary xylem elements which established connexion with the newly formed xylem elements of the host (Text-fig. 8, xxx). According to Barber (1907), most 'Self-attacks show very remarkable effort at resistance on the part of the Sandal root attacked', but in the instance just described there is no evidence of this.

In some instances the haustoria of *Santalum* fail to effect penetration into the host. An interesting example is furnished by the growth of an haustorium on a fruit of *Arachis hypogea*, where the thick pericarp prevented the sucker from reaching the seeds (Text-fig. 10). It did, however, succeed in penetrating the outer layers of the fruit-wall and in spreading for a considerable distance along the less resistant middle layer, rupturing the cross-bars of mechanical tissue on its way, but the internal layers of mechanical tissue prevented its penetration into the cavity of the fruit. The vascular bundles of the pericarp were tapped by the sucker. The outer surface of the haustorial cushion was thrown into numerous folds (Text-fig. 10, AA), while in the interior there were several collapsed layers which were irregularly undulated.

(b) *Osyris arborea*. The structure of the haustorium resembles that of *Santalum* in many respects. Certain features presented by the haustoria on the roots of *Botrychium virginianum* and on those of Monocotyledons, however, require mention. The root of *Botrychium*, being devoid of mechanical tissues, is easily penetrated by the haustorium; Pl. III, Fig. 5, shows clearly the pressure exerted by the grip of the clasping folds. As soon as the cortex of the host is ruptured, the sucker penetrates rapidly, while the tissues surrounding the nucleus fail to grow at the same rate, so that large lacunae form (Pl. III, Fig. 5). The central cylinder of the host is completely invaded, the pith being partly destroyed and partly pushed apart. The cells at the surface of the sucker that come in contact with the vascular tissue of the host are transformed into vascular elements, while others remain as glandular cells. The attacked root is sometimes completely destroyed, although the haustorium may remain intact for some time.

TEXT-FIGS. 9 and 10. Fig. 9. Longitudinal section of a young haustorium of *Santalum* on a rootlet of *Coffea arabica*. Note the rootlet of the host with root-hairs being split by the sucker, showing its vigorous action. M, vascular strand from the mother root; N, neck of the haustorium; P, pith; V, vascular cylinder of the haustorium seen as two strands in section. Note the swelling on the cylinder at vv, an indication of the pressure to which it is subjected while growing. A, clasping folds; BB, collapsed layers; S, sucker; GG, glandular cells; H, host-rootlet. ( $\times 55$ ). Fig. 10. Longitudinal section of an haustorium of *Santalum* on the fruit of *Arachis hypogea*. The sucker has been prevented from penetrating the fruit wall completely. H, haustorium; vv, vascular strands of the haustorium; AA, cortical folds. Haustorium and cortical folds are thrown into a number of folds on their outer margin, due to shrinkage or contraction. S, sucker which has penetrated the outer layer of the fruit wall F, and has sent penetrating lobes. SL, fruit wall; T, testa; E, seed. ( $\times 35$ )



TEXT-FIGS. 11-13. Fig. 11. Longitudinal section of fan haustorium of *Thesium* on a grass-root. The repeated attempts at penetration of the host-root can be seen in the formation of 5 successive pairs of cortical folds (1-5) and 5 nuclei (A-E) in the same order. M, mother root; N, neck of the haustorium. ( $\times 70$ .) Fig. 12. Longitudinal section of an haustorium of *Osyris arborea* on the grass-root. Two attempts have been made by the haustorium before penetration is effected. M, mother root; AA, two nuclei; 1 and 2, two cortical folds; B, collapsed layers of the cortical folds; H, host root completely surrounded by the cortical folds. Fig. 13. Transverse section of an haustorium of *Osyris* passing through the host root. 1, 2, cortical folds; AB, their collapsed layers; H, host root with vascular elements. ( $\times 90$ .)



(c) *Scleropyrum Wallichianum*. The genus *Scleropyrum* is confined to India, Burma, and the East Indies. Arnott (1838) does not mention its parasitic nature. *S. Wallichianum*, a small tree, 20–30 ft. in height, with conical axillary spines, has a well developed and deep root-system. The lateral rootlets produce haustoria whenever they come in contact with suitable foreign roots, and a single rootlet sometimes forms 3–4 lateral haustoria along its course (Text-fig. 1, H). The freshly dug haustoria are dull white and turgid; in the young ones the surface is smooth, while in the older ones it becomes rough, and concentric lines of growth appear.

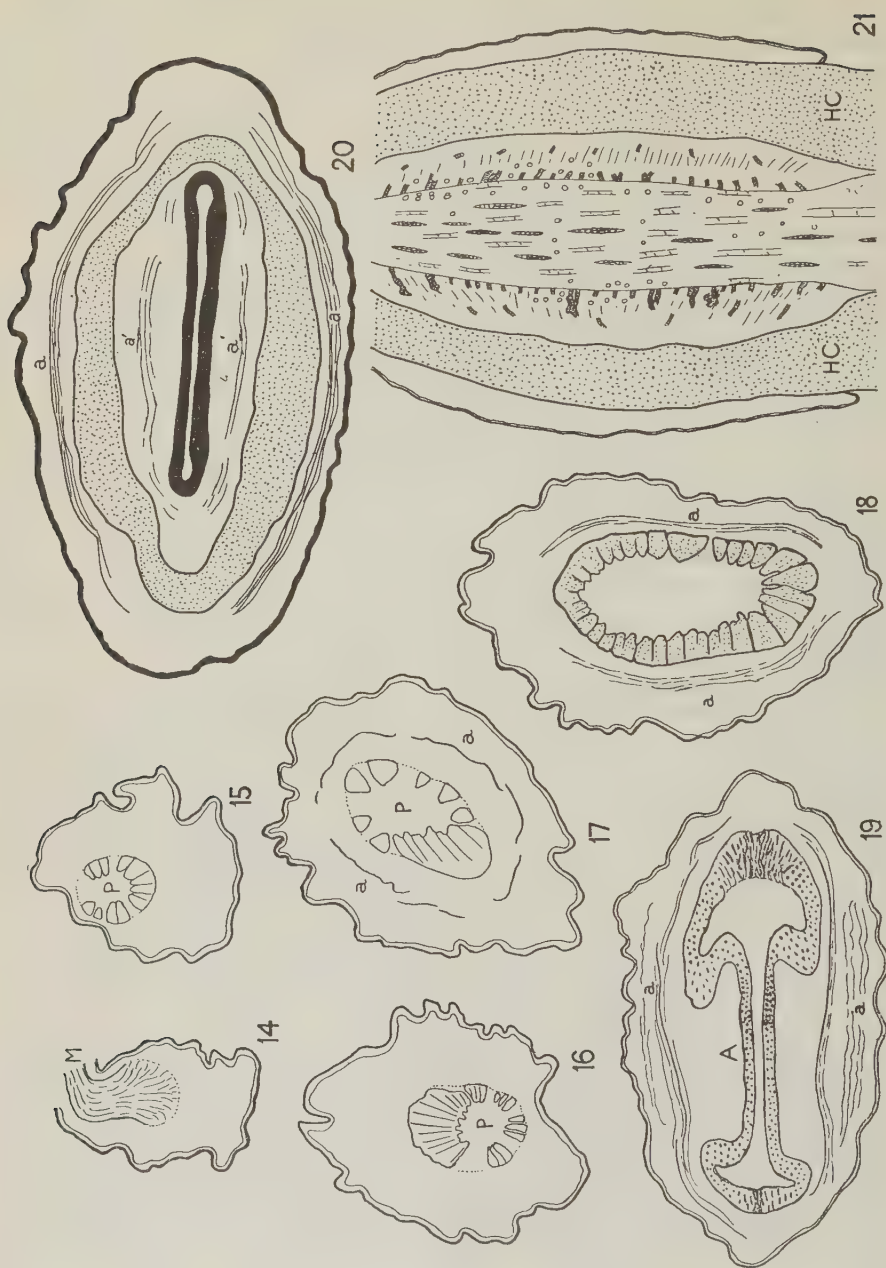
Hauustoria of *Scleropyrum* were collected on the roots of *Strobilanthes* sp.?, *Nephelium* sp.?, *Hopea parviflora*, *Memecylon indica*, *Cinnamomum zeylanicum*, *Terminalia paniculata*, *Schleichera trijuga*, *Pavetta indica*, and two species of *Ficus*. The texture of these roots varies from the relatively soft one of *Strobilanthes* to the fairly hard roots of *Terminalia*, which offered difficulties in securing good microtome sections.

The haustorium is generally conical in shape with a broad base, although it is sometimes compressed and more or less hemispherical, covering one-half to one-quarter of the circumference of the host root. The fully developed haustorium possesses three axes, one vertical passing from neck to apex and the other two horizontal; one of these, which is longer than the other, runs parallel to the axis of the host root, while the other is at right angles to it. The size varies according to age and the host attacked; the diameter at the base ranges from 3 mm. to well over 20 mm. along the long axis.

Sections of these haustoria were cut in different planes and are described as follows: a section cut at right angles to the vertical axis of the haustorium is transverse (Text-fig. 3, EF), while sections cut parallel to the latter are longitudinal (Text-fig. 2, AB, and Text-fig. 3, CD). In previous descriptions, which relate the direction of the section to the host, the longitudinal sections have been described as transverse, while the transverse section corresponds to the longitudinal sections of other investigators (Text-figs. 14–21).

In a medium longitudinal section passing along the short axis of the fully developed haustorium the vascular strands entering at the neck from the parent root and opening out to form the characteristic loop can be recognized, as well as the clasping folds and the penetrating sucker lifting the cortex from the underlying cambium. The sucker spreads for a considerable distance along the cambium. Penetration of the host is complete and the gland is replaced by parenchymatous tissue, although remnants of it are seen at the end of the sucker (Text-fig. 5, G). The place of the nucleus is taken by the central core of pith (Text-fig. 5, P). The former secretory action of the glandular cells can be recognized by the by-products found along the line of junction between the tissues of the host and parasite.

Regarding the course of the vascular strands in the haustorium of Santalaceae in general, there is divergency of opinion, which is perhaps due to the fact that chiefly longitudinal sections have been studied. Transverse sections



taken at different levels through the haustorium of *Scleropyrum* show that the vascular cylinder in the neck of the haustorium is constructed like that of a lateral root (Text-figs. 14–16). Beyond the neck it opens out and the component elements diverge to form the loop. The radial arrangement of the vascular strands gradually gives place to a bilateral one (Text-figs. 18–20), the degree of approximation of the two flanks progressively increasing. In the region of the loop the vascular cylinder is laterally compressed in the horizontal plane, the compression being more marked in the middle than at the ends (Text-fig. 19, A). The vascular cylinder remains compressed (Text-fig. 20), but in the sucker it opens out again; the component elements diverge and spread over a large part of the wood of the host (Text-fig. 21). The form assumed by the vascular cylinder at different levels, together with the occurrence of collapsed layers and their distribution, show the strains to which the haustorium is subjected in the course of its growth.

The opening effected by the sucker in the cortex of the host is not circular but oval in outline, which is a result of the shape of the sucker. The scars left by the decayed haustoria on older roots of the host are always oval or slit-like in outline. The relative positions of the raised cortex of the host root and the haustorial cortex can be clearly seen only in transverse section (Text-figs. 20 and 21).

Instances of self-parasitism are not uncommon in *Scleropyrum*. If the attacked root is very young, there appears to be complete fusion of tissues, and in one instance even the cortical layers of the two roots were fused, while the vascular strands of the parasite were diffused in the tissue of the haustorium and showed very few connexions with those of the host (Pl. III, Fig. 4).

(d) *Choretrum* and *Exocarpus*. The rootlets bearing the haustoria in *Choretrum glomeratum* are very slender and fragile. They creep along the

TEXT-FIGS. 14–21. Series of transverse sections of an haustorium of *Scleropyrum* on *Cinnamomum zeylanicum*, at different levels from the neck of the haustorium to its apex. These sections give a good picture of the disposition of the vascular cylinder throughout its length. ( $\times 28$ .) Fig. 14. Section at the place entry of the main vascular strand from the mother root. Fig. 15 and 16. Sections passing at a lower level than that of Fig. 14. Note the vascular cylinder with central pith (P) and radiating pith rays. There is a tendency for this radially symmetrical cylinder to become bilaterally symmetrical. Fig. 17. Section passing above the level of the loop. The vascular cylinder shows distinct signs of bilateral symmetry. The collapsed layers (a) are seen in the thick cortex of the haustorium. Fig. 18. Section passing at the level of the vascular loop or diverging vascular cylinder. Note the bilateral symmetry of the vascular cylinder and the collapsed cortical layers (a). Fig. 19. Lower down the vascular loop the cylinder exhibits two side pouches which extend only for a short distance down the haustorium. Fig. 20. Section passing at a level between the vascular loop and the sucker. The vascular cylinder is hollow and is very much compressed. The dotted area is the cortex of the host, which has been raised by the ingrowing sucker. Note the extension of the collapsed layers just outside the vascular cylinder. Fig. 21. A section passing at the level of the sucker where its xylem elements join those of the host. Dotted bands on the sides are the cortex of the host (HC); external to these are found the cortical folds of the haustorium. Inside the host cortex and its woody cylinder, the sucker of the haustorium joins the latter. Xylem elements of the sucker appear as reticulate vessels in surface view and as circles in sectional view. The glandular elongated cells are represented by black lines.



root of the host, producing minute lateral haustoria along their length. In this respect they resemble those of *Choretrum lateriflorum* described by Herbert (1920), who, however, was doubtful as to the penetration of the haustoria into the host.

In *C. glomeratum* the haustoria develop in the normal way, assuming a conical or sometimes hemispherical form, but the cortical folds are not well developed. After entry into the host the sucker penetrates deeply into its wood in the form of a wedge. The vascular loop and the spreading sucker, so characteristic of other *Santalaceae*, were not observed. The pith as usual replaced the nucleus and the gland in the fully developed haustorium. The vascular elements of the sucker are reticulately thickened vessels.

The haustorium of *Exocarpus aphylla* resembles that of other *Santalaceae* in the development of cortical folds with collapsed layers and the form of the vascular cylinder with its characteristic loop. The sucker penetrates deeply into the host-wood in the form of a wedge as in *Choretrum glomeratum*. The sucker of these two species is more like that seen in *Krameria canescens* (Cannon, 1910) than in other *Santalaceae* plants.

#### (ii) *Compound Haustorium.*

*Osyris arborea* and *Thesium Wightianum*. While simple haustoria are very common in *Osyris* and *Thesium*, compound haustoria are occasionally met with, especially in connexion with hard roots which are difficult to penetrate. Thus, on a hard root like that of a grass, where the mechanical tissues offer great resistance to penetration, the structure of the haustorium of *Osyris* becomes more complex. It develops an internal gland and may repeatedly form new clasping folds which completely encircle or envelop the host root. Finally the wedge-shaped sucker forces its way into the root (Text-fig. 12). The mechanical action of the sucker appears to be more pronounced in this case, as is shown by the splitting of the host root and the penetration of the vascular elements of the sucker to a greater depth into the vascular cylinder (Text-fig. 12). The successive efforts at penetration made by the haustorium are evident in the presence of two pairs of clasping folds (Text-figs. 12 and 13, 1 and 2), and two superposed nuclei (Text-fig. 12 A, A).

*T. Wightianum* does not differ appreciably in the structure of its haustorium from those of other species that have been investigated. Compound haustoria have, however, not hitherto been adequately studied. They appear to commence as simple haustoria and the compound character only becomes gradually established. The host root is completely encircled by the clasping folds. Five pairs of clasping folds were present in the haustorium investigated, the first pair being lifted upwards during the development of the later ones, which are progressively larger. It is the last formed functional folds that are actually in contact with the host and they are invariably better developed than the preceding ones (Text-fig. 11, 1-5). Sometimes smaller folds which are early suppressed arise between the larger ones; this is seen between 3 and 4 in



Text-fig. 11. When there are five nuclei formed in regular succession, the oldest is near the neck and the last functional one near the sucker. The root is finally encircled by the folds and split by the sucker which drives in like a wedge, as in the haustorium of *Osyris*.

On the basis of the classification of haustoria suggested by Fraysse (1905), those of *Osyris arborea* and *Thesium Wightianum* on the grass-roots are compound haustoria, while those of the former on *Botrychium* are simple haustoria. Morphologically, the compound haustorium is equivalent to a succession of simple superposed haustoria. In the compound haustoria there are usually as many pairs of cortical folds as there are embedded nuclei.

#### IV. DISCUSSION

The haustoria of Santalaceae are complex structures with two longitudinal planes of symmetry. In studying their structure their intimate association with the host must be taken into consideration. The sucker has generally been described as two-lobed and the xylem elements as occurring in two strands in all, except *Exocarpus* and *Comandra*. The bilobed or fish-tail appearance of the sucker and the arrangement of the vascular elements in two strands are based on the appearance obtained in longitudinal sections of the haustoria, but these do not afford a true picture. The same is true of the interrupted zone of the vascular cylinder described by Barber, which can only be interpreted correctly by a study of sections passing through different planes. The vascular cylinder has the shape of an inverted flask, the mouth of which is in the region of the sucker, and it is continuous with that of the parent root in the neck.

There is some difference of opinion as to the nature of the xylem elements in the haustorium and their mode of attachment to those of the host. Kusano (1902) states that the vessels in *Buckleya quadrila* are short and have oblique end walls and that they are reticulate, rarely pitted. According to him few vessels abut directly on the wood of the host—and these come in contact with the radial walls of the xylem elements. Moss (1926) states that the vascular system of the haustorium of *Comandra* consists mainly of tracheides with reticulate thickenings and that these ordinarily become very intimately associated with the xylem of the host. He records the occasional penetration of the xylem elements of the latter. Barber (1907) also describes vascular elements with reticulate markings and concludes that the elements of the host and of the haustorium form practically one tissue, although a line of separation is indicated by a layer of yellowish secretion. Fraysse (1905) found the xylem elements of the haustorium of *Osyris alba* in intimate contact with the vessels of the host, although there was no penetration. This study has shown that the xylem elements of the haustoria are true vessels, and that connexion with the xylem elements of the host is brought about by apposition of the pit-apertures of the elements concerned and not by penetration. When the end wall of a

xylem element of the sucker comes in contact with the xylem elements of the host, the pits are so adjusted that corresponding ones lie opposed to one another. It must be remembered in this connexion that tignification of the xylem elements of the sucker takes place, after it has become attached to the xylem of the host, so that adjustment of the pits is easy.

The haustoria are capable of developing secondary tissues, if the circumstances are favourable. A cambial layer is always recognizable, although many of the haustoria do not produce secondary tissues and thus do not become permanent. In nature the transformation of an haustorium into a permanent structure by secondary growth depends upon many factors which are chiefly biological. The nature of the host and its degree of tolerance of the parasite appear to be important, as shown by the haustorial relation between *Santalum album* and the host *Jasminum*, and the instances of self-parasitism described in *Santalum* and *Scleropyrum*.

The mechanical action of the haustorium on the grass-root appears to be more pronounced than the chemical, as seen in the compound haustoria of *Osyris* and *Thesium*, in which the host root is invariably split by the invading sucker. This seems to be partly due to the predominance of unyielding mechanical tissue in the host root and partly to its high resistance to the solvent action of the secretion. The complete absence of such splitting in the dicotyledonous roots, which possess softer tissues, implies that they are structurally better suited to serve as a host. It would also appear that compound haustoria are formed whenever the host tissues are difficult to penetrate and that their development is not related to the family to which the host belongs. This is also suggested by the instances of compound haustoria mentioned by Kusano (1902) in *Eucleya* and Barber (1907) in *Santalum* which are developed on coniferous and dicotyledonous hosts respectively.

## V. SUMMARY

The root-parasitism of *Santalum album*, *S. lanceolatum*, *Thesium Wightianum*, *Osyris arborea*, *Exocarpus aphylla*, *Choretrum glomeratum*, and *Scleropyrum Wallichianum* has been studied for the first time.

The haustoria of Santalaceae differ considerably from those of Rhinanthaceae and Balanophoraceae, but only slightly from those of Olacaceae.

The haustoria may be simple or compound; the latter consist of a series of superposed simple haustoria and are generally found on hard roots which are difficult to penetrate. In both there is glandular activity in the surface layers of the haustorium, but where this fails to effect penetration, an internal gland is developed; after it has fulfilled its function, it is replaced by other tissues.

There is a direct vascular connexion between the parent root and the host via the haustorium. The vascular cylinder of the mature haustorium has the form of an inverted flask. The xylem of the haustorium consists of vessels which become apposed to those of the host by their pit-apertures.

The sucker penetrates the cortex of the host, and raises it along the cambial layer.

Secondary growth may occur in the haustorium which thereby becomes a permanent structure and may then function for a long time. Most of the haustoria are short-lived.

Self-parasitism has been observed in *Santalum* and *Scleropyrum*. In the latter complete fusion of tissues of the host and parasite takes place.

My thanks are due to Prof. T. G. B. Osborn, who kindly passed on to me specimens of haustoria of *Santalum lanceolatum*, *Exocarpus*, and *Choretrum* from his Australian collections. I am also grateful to Prof. H. G. Champion, and to Dr. L. Chalk for giving me facilities at the Imperial Institute of Forestry, Oxford, to carry on this investigation. I have to thank Prof. F. E. Fritsch for his valuable criticisms and suggestions in completing this investigation.

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### EXPLANATION OF PLATE III

Illustrating the article by L. N. Rao on 'Parasitism in the Santalaceae'.

FIG. 1. Longitudinal section of young haustorium of *Santalum album* on the rootlet of *Coffea arabica*, which has been split by the sucker. Collapsed tissue in the form of dark streaks can be seen on both sides of the nucleus. A second pair of clasping folds are just being formed. The flask shape of the vascular cylinder is obvious. ( $\times 40$ .)

FIG. 2. Internal glandular apparatus of a sandal haustorium on the root of *Dodonaea viscosa*, showing its position in relation to the haustorium. The beginning of the collapsed layer is also seen. ( $\times 20$ .)

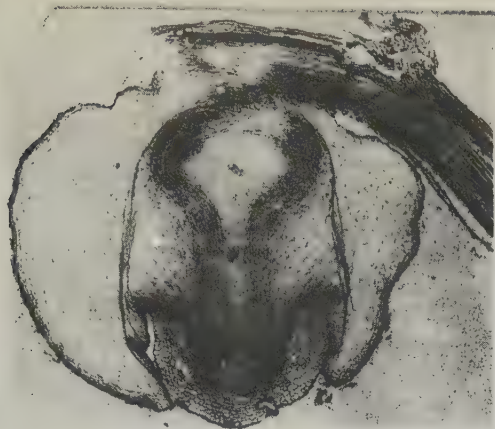
FIG. 3. Scleropyrum on the root of *Ficus* sp.? Note the vascular loop and the highly spreading sucker, which lifts up the cortex of the host. The glandular activity is increased at the points where the lifted up cortex comes in contact with the cortical folds. ( $\times 14$ .)

FIG. 4. Self-parasitism of Scleropyrum. The host and the parasite roots have completely fused and the vascular system of the parasite is very poorly developed as scattered isolated strands, while that of the host root is somewhat displaced by the growing in of the parasite tissue. This is unique in showing complete fusions of tissues. ( $\times 15$ .)

FIG. 5. Longitudinal section of an haustorium of *Osyris arborea* on a root of *Botrychium virginianum*. The host root has been pierced by the sucker whose glandular cells are in contact with the cells of the root. The clasping folds, the collapsed layer with prominent lacunae, and the vascular strand coursing down from the mother root are seen. ( $\times 28$ .)

FIG. 6. Longitudinal section of an haustorium of *Santalum lanceolatum* on *Acacia*, the parent root at the top in transverse section; the course of the vascular cylinder, and the sucker inside the host are seen. ( $\times 10$ .)

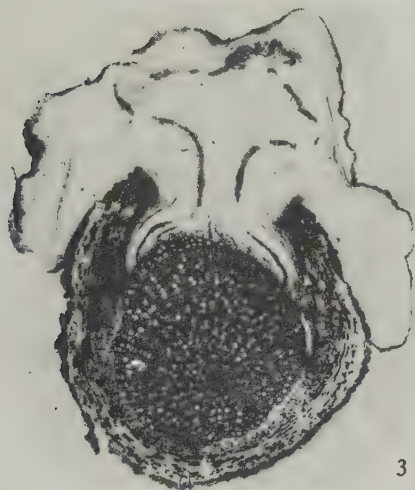




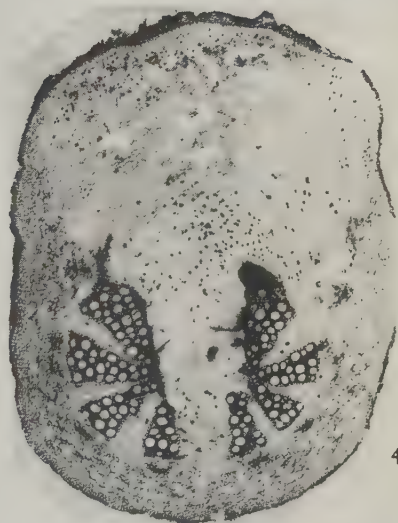
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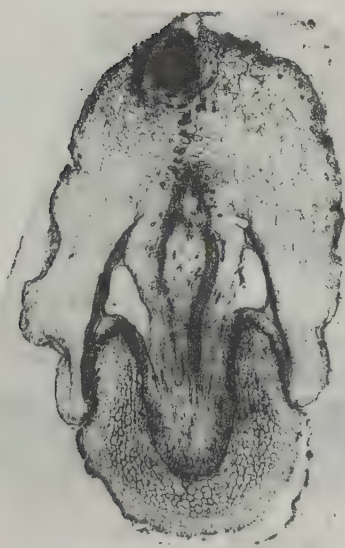
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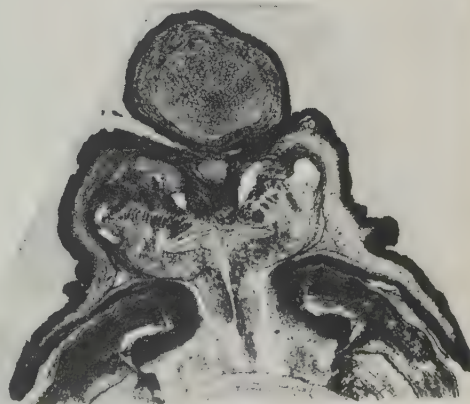
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Huth, Stubbs X., Kent.



# Studies in the Santalaceae

BY

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With Plate IV and fifty-seven Figures in the Text

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## INTRODUCTION

THE earliest account dealing with the embryology of Santalaceae is that of Griffith (1845), who described in *Santalum album* the outgrowth of the embryo-sac, into the cavity of the ovary, towards the pollen tube. The nature of the egg-apparatus was then unknown, so that Griffith's remaining conclusions do not conform to present-day ideas. In a second paper (1845) he mentions the posterior prolongation of the embryo-sac, but the other details he gives are of necessity limited by the technical facilities then available. Henfrey (1856), working on *Santalum album*, confirmed some of Griffith's observations. Schacht (1866), studying preserved material of *Santalum album* from the Calcutta Botanic Garden, describes the development of the embryo-sac correctly up to a certain stage, but he mistook the two synergids for eggs and his conclusions on fertilization and embryogeny are incorrect. Jönsson (1879) gave a rather incomplete account of the development of the embryo-sac in *Thesium intermedium* and *Osyris alba*. Guignard's (1885) work on the development of the embryo-sac in *Thesium humifusum*, *T. alpinum*, *T. divaricatum*, and *Osyris alba* remained the standard one for a long time. Strasburger (1885) gave a detailed account of the embryo-sac of *Santalum*

*album*. More recently Modilewski (1928) has described the early developmental stages of the sac in *Thesium intermedium*, Schulle (1933) published an account of the life-history of *T. montanum*, and Iyengar (1937) described certain stages in the life-history of *Santalum album*. It is therefore clear that the study of Santalaceae has been confined mainly to the development of the embryo-sac. Species of the cosmopolitan genus *Thesium* have been chiefly investigated, while the economically important members have been ignored and there are still wide gaps in the knowledge of their life-history. The object of this research is to fill these gaps and at the same time to study the Indian representatives of Santalaceae. According to Pearson and Brown (1932), the Santalaceae are represented in India by 8 genera and 15 to 20 species; 4 belonging to different genera are dealt with in this investigation.

#### MATERIAL AND METHOD

The material was fixed in the field in different parts of India, the principal fixatives used being medium chrome-acetic with osmic and Bouin fluid. After fixation the material was treated in the usual way and finally embedded in paraffin. Sections were cut of different thickness suited to the nature of the structure to be investigated: they were stained with Haidenhain's iron-alum-haematoxylin differentiated with acid alcohol, Newton's gentian violet, safranin with light-green, safranin with anilin blue, while some preparations were stained with haematoxylin and gentian violet, a new combination found useful in the study of the embryo-sac haustorium. Some were mounted in glycerine jelly, which shows up certain structures very well, while the majority were mounted in balsam.

#### (i) *Mitosis*

#### CYTOLOGY

Mitosis was studied in the cells of the root-tip. The only feature of interest is the behaviour of the phragmoplasts during cytokinesis. At early anaphase the spindle striations are very faint, but with the onset of telophase they gradually become more and more stainable, especially in the region between the future daughter nuclei. This is probably due to the accumulation of spindle-forming substance or phragmoplast along the striations. This accumulation commences in the axial region of the spindle and gradually extends towards its periphery; the peripheral extension is connected with the centrifugal growth of the new partition wall which originates in the axial region, midway between the daughter nuclei (Text-fig. 1). As the phragmoplasts move towards the periphery of the spindle they appear to form a hollow cylinder open at both ends, where the daughter nuclei are situated. At a later stage the barrel becomes very much dilated in the middle until it touches the side wall. At this stage (Text-fig. 2) the phragmoplasts can be clearly seen, in optical section, as two groups of highly staining and closely apposed rods which are situated on either side of the cell and are connected by the thin



partition wall. Later the phragmoplasts disappear as the growth of the partition wall is completed. The phragmoplasts appear to play an important part in the formation and growth of the division wall (Text-fig. 3). The



TEXT-FIGS. 1-16. Figs. 1-11. *Santalum album*. (×2,000.) Fig. 1. Division of cell of root-tip; phragmoplasts prominent along the spindle-striations. Fig. 2. Polar view of the preceding showing the daughter nuclei at different levels. Around them the phragmoplasts form a ring of uniform thickness. Fig. 3. As the division wall is formed the phragmoplasts move towards the periphery of the spindle. A stage in this movement. Fig. 4. Chromosomes on the metaphase plate from a root-tip of *Santalum*. Figs. 5 and 6. Stages in diakinesis. Fig. 7. Nucleolar attachments shown on a large scale. Fig. 8. Bivalents on the meiotic metaphase plate. Fig. 9. Metaphase plate (side view). Fig. 10. Anaphase (side view). Fig. 11. Telophase. Note the shape of the spindle and the special wall between the cytoplasm and the mother-cell wall. Figs. 12-14. *Thesium Wightianum*. (×2,000.) Fig. 12. Somatic chromosomes from the cells of young embryo. Twenty chromosomes are found. Fig. 13. Bivalents on metaphase plate. Ten bivalents are seen. Fig. 14. Cytokinesis by ingrowths from the special wall to the centre of the mother-cell, cutting the cytoplasm into four parts. Figs. 15 and 16. *Osyris arborea*. (×1,350.) Bivalents on the metaphase plate. Fifteen bivalents are seen.

association of the cell plate with the formation and lateral movement of phragmoplasts has been observed by Sharp (1911) in *Physostegia virginiana*. The same author (1934) is of the opinion that a portion of the spindle

substance re-enters the daughter nuclei, while the remainder functions as phragmoplasts.

The somatic chromosome number is twenty. The chromosomes are short and oval in shape (Text-fig. 4).

Mitosis was studied in the developing embryo of *Thesium*. The chromosomes are curved and rod-shaped and the somatic complement is twenty (Text-fig. 12).

## (ii) *Meiosis*

The numerous pollen mother-cells are polygonal and closely packed in the locule, being arranged in 6 to 10 longitudinal rows. The cytoplasm of the mother-cell is fibrillar, while the large spherical nucleus occupies a quarter to a half of the volume of the cell. The fibrillar cytoplasm is denser around the nucleus, giving the appearance of a perinuclear zone. The nucleus contains a prominent nucleolus and a fine meshwork on which chromatin granules are situated. At the beginning of prophase the cytoplasm of the mother-cell contracts slightly away from the walls, first at the corners and later on all sides. This phenomenon is in all probability due to the formation of a 'special wall' between the cytoplasm and the mother-cell wall. The mother-cells gradually become spherical and separate from one another. As the prophase advances the nucleus enlarges and the network condenses, while a parallel arrangement of the threads becomes recognizable. They shorten and become less coiled and pass on to the diakinesis stage (Text-figs. 5 and 6). The bivalents lie free in the cytoplasm owing to the disappearance of the nuclear membrane and of the nucleolus. Ten bivalents were counted on the metaphase plate (Text-figs. 8, 9, 10). The chromosomes are very small, so that the chiasmata are difficult to recognize. After the organization of the daughter nuclei there is a period of rest of varying duration. The spindles of the second division are never parallel. After the four daughter nuclei are formed cytokinesis begins. Iyengar (1937) describes cytokinesis in *Santalum album* as taking place by peripheral constriction of the cytoplasm, midway between the nuclei, these constrictions extending inwards until they meet at the centre. Careful observation reveals the presence of a 'special wall' which reaches its maximum thickness at the end of the second division (Text-fig. 11). It easily escapes observation owing to its hyaline nature. When the four daughter nuclei are organized, conical protrusions appear on the special wall midway between the nuclei; they extend inwards and ultimately divide the cytoplasm into four parts. Cytokinesis is thus effected primarily by ingrowths from the special wall and cytoplasmic constriction is secondary.

Meiosis in *Thesium Wightianum* does not differ much from that of *Santalum*. Ten bivalents were clearly made out at diakinesis, two of them being attached to the nucleolus. The same number of attachments was observed at the prophase stage. Ten is the haploid chromosome complement (Text-fig. 13). Cytokinesis takes place as in *Santalum* (Text-fig. 14). Germination of

the pollen tube is normal, the generative nucleus dividing in the pollen tube to form the two male nuclei (Text-fig. 40).

In both male and female flowers of *Osyris arborea* pollen mother-cells develop and undergo meiosis, but by the end of the second division the microspores in the anthers of the female flowers are arrested. The mother-cell usually hypertrophies and ultimately degenerates. If microspores are formed, all the spores of a tetrad are not of the same size, and they, too, ultimately degenerate. The nucleus of the mother-cell is large, with one or two nucleoli. Two nucleolar attachments are visible in prophase. Fifteen bivalents are counted at diakinesis and in metaphase (Text-figs. 15 and 16). Cytokinesis is associated with formation of a special wall as in *Santalum*.

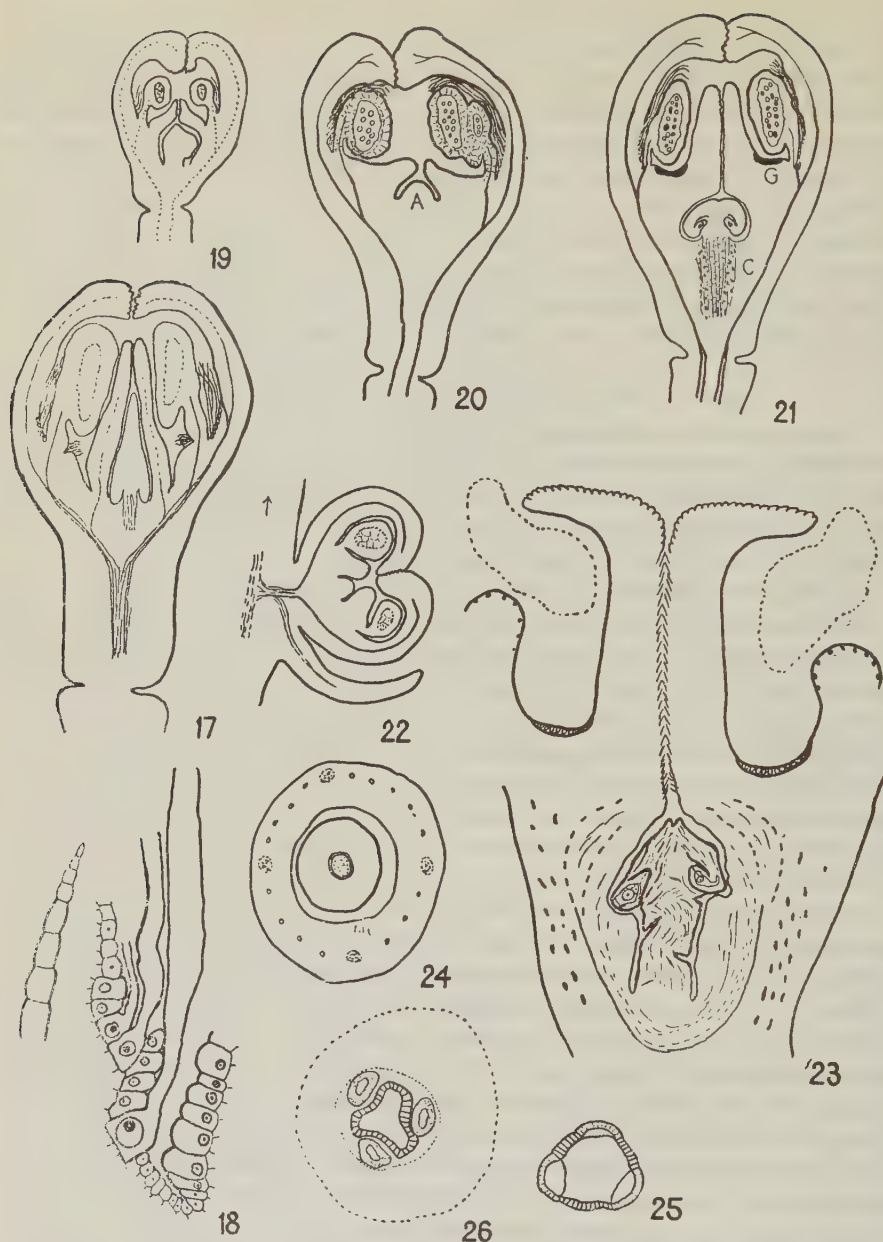
#### DEVELOPMENT OF THE FLORAL PARTS

The order of development of the floral parts is similar in all four species. The first part to differentiate is the perianth, then the androecium, and then the gynaecium, while the central placenta or 'mamelon' appears last. By the time that the carpels begin to develop the perianth encloses the other parts of the bud. The tips of the carpels meet to enclose the ovary cavity.

In *Santalum album* the floral axis forms a conical projection into the completed ovary cavity and upon it three ovules differentiate in a basal or slightly lateral position. The placenta rapidly enlarges as the result of basal and intercalary growth, so that it fills the small cavity of the ovary and becomes compressed and contorted in adjusting itself to the limited space available. Meanwhile the ovules enlarge and become pendulous, ultimately dipping into three depressions around the base of the placental column. During the development of the flower the gynaecium becomes semi-inferior or inferior as a result of partial fusion between the carpellary walls and the perianth (Text-fig. 17).

The bisexual flowers of *Santalum album* are generally tetramerous, although occasionally pentamerous. Transverse sections through different levels of a tetramerous bud indicate the relative positions of the floral parts (cf. Text-figs. 24–26). A section passing through the anthers shows unicellular hairs with an enlarged base containing a prominent nucleus (Text-fig. 18) between them and the perianth segments; these are called staminal hairs by Ewart (1892) in *Thesium*, but they are borne on the perianth segments and have no connexion with the stamens. The filaments in *Santalum album*, however, bear, in addition, a group of short unicellular hairs which are directed towards the centre of the flower (Text-fig. 17). Other Santalaceae do not possess these staminal hairs. Sections passing through the bases of the filaments show four glands, alternating with the stamens and perianth. They do not contain a vascular strand like the other floral parts, so that there is nothing, except their position, to prove that they are modified petals (Schacht, 1866). They appear to have a secretory function as indicated by the structure of the cells.





TEXT-FIGS. 17-26. Fig. 17. Longitudinal section of an unopened flower of *Santalum album*, showing the relative positions of the floral parts. The glands are not shown. Note the groups of hairs on the filaments. ( $\times 35$ .) Fig. 18. Staminal hairs of *S. album* with their basal portions containing large nuclei and forming part of the perianth. Terminal portions of the hairs show the constrictions. ( $\times 200$ .) Fig. 19. Section of a young flower-bud of *Thesium Wightianum*. Elongation of the placental column and development of the ovules



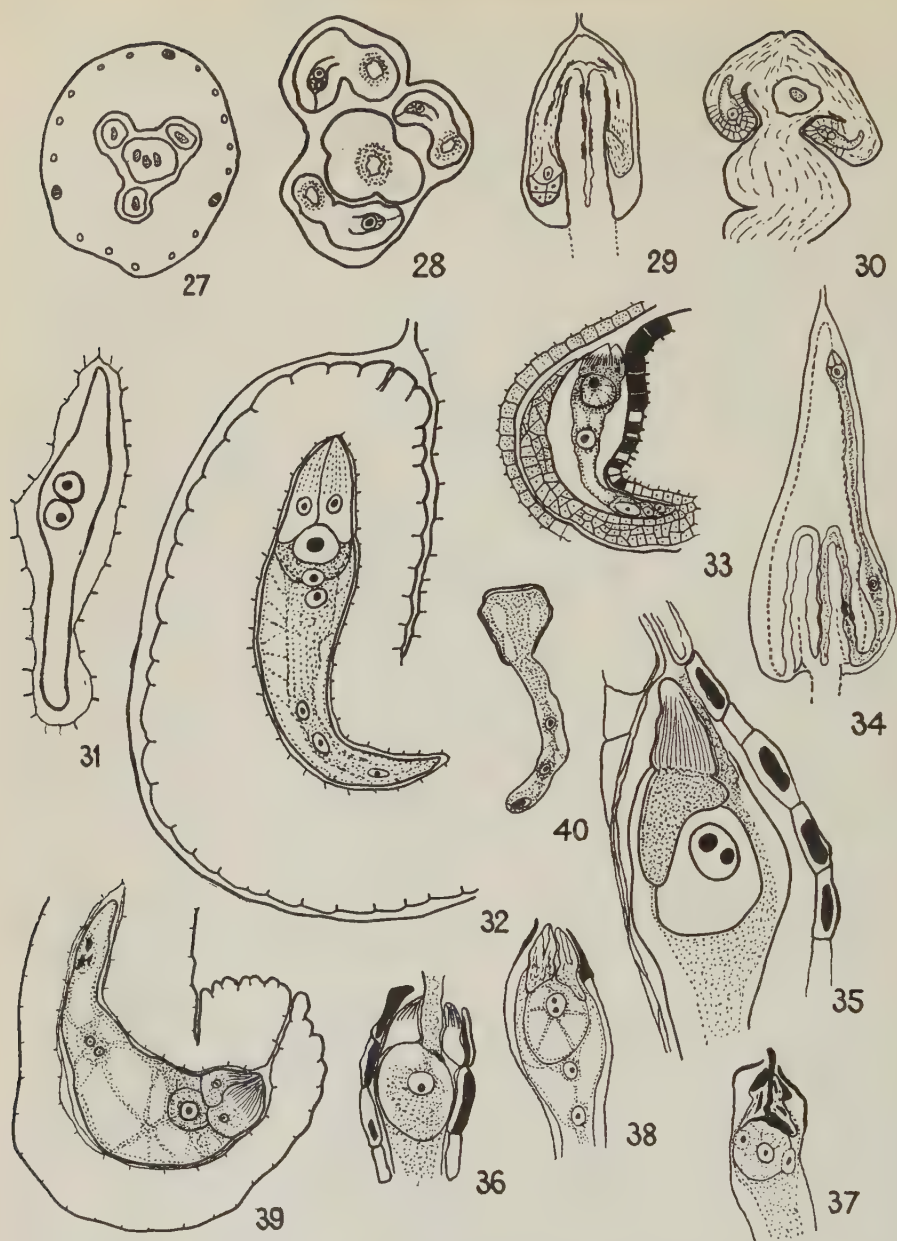
Sections at the level of the ovary show the placental column with surrounding ovary wall (Text-fig. 24) and the vascular strands supplying the floral parts. Text-fig. 25 shows the placental column cut at a higher level with the ovules partly fused with it, whereas in Text-fig. 26 they are free. In a section through the base of the ovary it appears trilocular, although higher up it is unilocular. On these characters Troll (1928) describes such gynaeceia as 'paracarpous'. Text-fig. 24 shows the vascular strands of the flower-stalk diverging to pass into the perianth; the four large strands enter the perianth segments after giving off branches to the stamens, while the smaller strands supply the carpels. The placental column, whose base is just above the place where the main vascular strands diverge, does not appear to receive any vascular trace from the main floral strands.

In *Thesium Wightianum* the independent origin of the placental column and ovules is clearly recognizable. The former does not receive any vascular tissue from the basal strand from which all the other floral parts are supplied (Text-fig. 19). The placental column becomes twisted and tortuous while accommodating itself to the cavity of the ovary, but it never undergoes fusion with the ovary wall. The mature ovules do not extend to the floor of the ovary as in *Santalum*, but bend up before reaching the base of the placental column (Text-fig. 29). There are no distinct glands, although the floral receptacle is glandular around the base of the stamens. Unicellular hairs are borne on the inner surface of the perianth, behind each stamen, and are so long that they arch over the anthers; their conspicuous basal portions are full of cytoplasm and contain large nuclei. In transverse section the ovary is three-lobed, the lobes harbouring the pendulous ovules. The wall is traversed by three large vascular strands, with smaller ones equally distributed between them (Text-fig. 27).

In *Osyris arborea* development proceeds in the same way until the differences between the male and female flowers become apparent. The placental column develops as in *Thesium*, but in the male flowers the ovules are usually

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are seen. Dotted lines show the course of the vascular bundles. The placental column does not receive a vascular trace. ( $\times 35$ .) Fig. 20. Longitudinal section of a male flower of *Osyris arborea*. A = undeveloped placental column. Vascular supply to the floral parts does not enter the placenta. ( $\times 35$ .) Fig. 21. Longitudinal section of a female flower of *O. arborea*. The pollen grains are degenerating in the anthers. The ovary is well developed with pendulous and upturned ovules. C = elongated cells below the placenta which become a conspicuous axial group or strand. G = glandular areas. ( $\times 35$ .) Figs. 22-3. *Scleropyrum Wallichianum*. Fig. 22. Longitudinal section of a young male flower showing the development of the floral parts. ( $\times 35$ .) Fig. 23. Longitudinal section of a female flower, showing the placenta with the ovules, the endocarp showing signs of growth, the style and stigma. Dotted areas represent the degenerated anthers; below them the floral glands with black dots indicating the position of stomata. ( $\times 35$ .) Figs. 24-6. *Santalum album*. Fig. 24. Transverse section passing below the base of the stylar canal showing the ovary wall with the vascular strands and the placental column with its axial elongated cells shown as (a) dotted area. ( $\times 35$ .) Fig. 25. Transverse section of the placenta showing the ovules fused with it. Fig. 26. Transverse section of the placenta at a lower level than that of Fig. 25, showing the pendulous ovules free from it. ( $\times 40$ .)



TEXT-FIGS. 27-40. Fig. 27. Transverse section of the ovary of *Thesium Wightianum* showing the vascular strands in the ovary wall and the way in which the pendulous ovules are lodged in the diverticula of the ovary cavity. The embryo-sac in the ovule and the chalazal haustoria in the placenta are shown as dotted areas. ( $\times 35$ .) Fig. 28. Transverse section of the ovary of *Scleropyrum*, showing the arrangement of the pendulous ovules around the placental column. Dotted areas around the haustorial tube show the layers of cells rich in

not produced, the central column remaining as a hemispherical knob, which is not covered by the carpels above (Text-fig. 20). The placental column does not receive a vascular strand from the main system of the flower. There are no glands, although small areas around the insertion of the stamens and around the base of the style consist of glandular cells. Hairs are found in groups behind the stamens in both flowers.

The male flowers of *Scleropyrum* are in catkins which are solitary or in groups on leafless spiny branches (Pl. IV, Figs. 1, 4), while the female flowers are produced on solitary, more or less pendulous, spikes (Pl. IV, Figs. 2, 3). The flowers are generally pentamerous. In the male flower the bracts appear first, while the development of the ovary and placenta is suppressed at an early stage (Text-fig. 22); in the female flower stamens are not well developed. The growth of the placental column is more rapid in the female flower than in *Thesium*, so that it becomes more distorted and fills the cavity of the ovary. The more or less pendulous ovules are comparatively small and do not reach even half the length of the placenta (Text-fig. 23). They curve inwards at an angle to the axis of the placenta (Text-fig. 30). The stamens differ from those of other Santalaceae in being bifid, each branch carrying a half anther.

The placental column remains free in *Santalum*, *Thesium*, and *Osyris*, while in *Scleropyrum* it fuses later with the ovary wall. A bundle of elongated cells with dense cytoplasm forms a conspicuous structure in the torus, being in continuation with the basal end of the placental column, especially in *Osyris* (Text-fig. 21). These cells appear to help in conduction during earlier stages, while subsequently some of them are transformed into irregularly distributed, discontinuous vascular elements, mostly vessels. The cells of the torus surrounding the bundle of elongated cells are rich in starch and

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storage material. ( $\times 60$ .) Fig. 29. Longitudinal section of the placenta of *Thesium* showing the pendulous ovules, one of them with the endosperm-haustorium cell while the other is bent upwards. The placenta shows the chalazal haustorium. ( $\times 60$ .) Fig. 30. Longitudinal section of the placental column of *Scleropyrum* showing how it is distorted and also the pendulous ovules with their tips turned upwards. ( $\times 60$ .) Fig. 31. A stage in the binucleate embryo-sac of *Santalum*. ( $\times 675$ .) Fig. 32. The upturned tip of the ovule of *Osyris*, showing the embryo-sac with its full complement. The synergids are just below the surface. ( $\times 450$ .) Fig. 33. Later stage of embryo-sac of *Osyris*. The polar nuclei have fused and the sac has emerged from the nucellus, so as to push the synergids slightly into the ovarian cavity. ( $\times 210$ .) Figs. 34–8. *Santalum album*. Fig. 34. Longitudinal section of the placenta with two ovules; one of them shows an embryo-sac with its full complement and the position occupied by the sac in the ovary. ( $\times 35$ .) Fig. 35. Side view of an egg-apparatus in which the male nucleus has fused with the egg-nucleus. One of the synergids is intact. ( $\times 450$ .) Fig. 36. The pollen tube has come in contact with the egg-cell and the two synergids are not disorganized. ( $\times 250$ .) Fig. 37. A later stage in fertilization. Synergids are disorganizing. One of the male nuclei has entered the cytoplasm of the egg, while the other is lower down, just touching it, perhaps on its way down to meet the fusion nucleus. The pollen tube is shown as a dark mass. ( $\times 260$ .) Fig. 38. Surface view of the fertilized egg, below which are two endosperm nuclei which have moved up from below. ( $\times 250$ .) Figs. 39 and 40. *Thesium Wightianum*. Fig. 39. The terminal portion of a pendulous ovule with its upturned apex, showing the micropyle and a fully organized embryo-sac. ( $\times 250$ .) Fig. 40. Germination of the pollen grain, with its tube nucleus and two male nuclei. ( $\times 450$ .)



other reserve materials, which form the chief source of supply to the haustorium. During its growth within the placenta the latter always follows the course of the vascular elements, destroying them on its way, and finally it takes over their function. The absence of a well developed vascular system in the placenta is perhaps connected with its subsequent replacement by the antipodal haustorium of the embryo-sac.

#### MEGASPOROGENESIS AND EMBRYO-SAC DEVELOPMENT

The development of the female gametophyte is similar in all four species. The hypodermal mother-cell gradually becomes embedded deep in the tissue of the nucellus. The embryo-sac is eight-nucleate. It always grows out of the ovule, either into the cavity of the ovary or into the placental column or both, and in its fully grown condition has the shape of the letters U or N.

In the young hemispherical ovules of *Santalum* one (sometimes two) of a central group of subepidermal cells enlarges to form the mother-cell which grows more in the longitudinal than in other directions. The reduction division is followed by the formation of a linear tetrad of which the micropylar member becomes the functional spore. This elongates to 3 to 4 times its original length and during this the binucleate condition is attained (Text-fig. 31).

As a result of further rapid elongation the micropylar end of the sac harbouring one of the two nuclei emerges from the nucellus and enters the cavity of the ovary. This prolongation of the sac curves upwards and grows within the ovary cavity, closely following the surface of the ovule or the placental apex. Meanwhile the nucleus within it has divided twice, and an egg-apparatus consisting of two synergids and an egg-cell almost touching them is organized at the distal end of the prolongation (Text-fig. 34), while the fourth (polar) nucleus moves down the sac. The other nucleus, which remained at the chalazal end within the ovule, divides into four, three of which constitute antipodals (Text-fig. 41), while the fourth moves out of the sac into the bend of the micropylar prolongation to meet the other polar nucleus from the micropylar end with which it later fuses. The bend in the prolongation of the sac is wider than the rest and sometimes appears to show outgrowths or branches (Henfrey, 1856), but these are only folds resulting from the accommodation of the prolongation to the available space within the ovary. By the growth of the prolongation the egg-apparatus comes to lie next to the placenta, very near its apex (Text-figs. 50 and 51).

The chalazal end of the sac continues to grow, while the antipodal nuclei remain at the side. The antipodal end of the sac penetrates the ovular tissue and passes upwards into the apex of the placental column, subsequently curving and growing downwards again for some distance. The fully developed embryo-sac thus has the form of an N, with the free ends much prolonged, the one occupying the tissue of the ovule and the placental column and the other the cavity of the ovary. The egg-apparatus, the two polar nuclei



in the dilated portion of the micropylar prolongation, and the three antipodal nuclei in the middle of the sac constitute its full complement in the mature condition. All three embryo-sacs of an ovary may reach maturity, although usually only one finally develops an embryo.

In *Thesium Wightianum* the hypodermal megaspore mother-cell appears within the ovular prominence before the latter begins to curve downwards. By the time the megaspore is formed the nucellar tissue above it has grown rapidly, so that it is deeply embedded, although a narrow passage ('micropyle') is left for communication with the ovarian cavity (Text-fig. 39). There are differences of opinion as to the nature of this micropyle. The formation and growth of the micropyle synchronizes with the upward curvature of the pendulous ovules; a similar curvature is shown by the embryo-sac and the endosperm. At the time of the first division of the nucleus the sac is elongated, slightly curved, and already shows enlargement at its micropylar end. One of the nuclei moves into the latter, while the other passes to the narrow chalazal end. When each nucleus has divided into four, the swelling of the micropylar end of the sac has reached its maximum, while the opposite end shows signs of growth in a backward direction. One nucleus of each group (polar nuclei) passes towards the middle of the swollen end, while the other three give rise to egg-apparatus and antipodal nuclei at the micropylar and chalazal ends respectively. The synergids have a pointed beak with a filiform apparatus, and the egg-cell lies close to them, partly covered on one side by their basal portions. The antipodal nuclei play no important part, but persist for some time and, even after fertilization, can be seen adhering to one side of the growing end of the sac. The polar nuclei remain closely apposed for a long time before fusing. Two mother-cells are occasionally met with, but twin embryo-sacs were not observed. It is noticeable that the embryo-sac of this species never emerges from the nucellus.

In *Osyris arborea* the single hypodermal mother-cell develops as in *Thesium*. The female prothallus develops normally, but while this is taking place the anterior end of the sac grows upwards and comes to lie close to the epidermis of the ovule (Text-fig. 32). The synergids show a prominent filiform apparatus and project slightly into the ovary cavity (Text-fig. 33), while the large egg-cell touches their basal walls. The polar nuclei remain close together and near the egg-cell for some time before fusing. As a result of further growth of the micropylar end of the sac the egg-apparatus is carried out of the ovule and just projects into the ovary cavity (Text-fig. 33). The antipodal cells persist after fertilization, but play no important part. Sooner or later, the chalazal end of the sac grows past the antipodal cells and penetrates into the placental column as far as its base.

In *Scleropyrum Wallichianum* the mother-cell soon becomes embedded, though a narrow passage (micropyle) is left, as in *Thesium*. The functional megaspore appears to be the distal one of the tetrad. At the eight-nucleate stage the embryo-sac has the form of a slightly bent cylinder, enlarged at the

region of the bend, and with narrow chalazal and somewhat narrow and blunt micropylar ends. The polar nuclei move to the widest part of the sac and lie close together. The synergids are relatively small and, though they possess conical apices, a filiform apparatus is not evident. The large egg-cell is situated below them. The micropylar end of the sac does not grow out of the nucellus, whilst the chalazal end penetrates the placental column, even before fertilization. The antipodal nuclei lie free in the cytoplasm and, after fertilization, move into the antipodal extension of the sac, where they fulfil an important function.

#### POLLINATION AND FERTILIZATION

Germination of the pollen grains on the stigmatic surface has been observed in nature in all species, and in *Thesium* germinating pollen grains have been found in the undehiscent anthers. Owing to the delicate nature of the pollen tube of *Santalum album* and the tortuous course followed in the stylar canal, it is difficult to trace it down to the embryo-sac, although it is easily distinguished near the apex of the latter, after it has left the conducting tissue of the style. By this time the apical portion of the embryo-sac membrane has disorganized and the synergids project into the cavity of the ovary. On coming in contact with the synergids the pollen tube appears, at least in some cases, to exert a certain pressure, as indicated by the expansion of its tip. It sometimes passes between the synergids, which do not completely cover the egg, and in such instances the latter remain intact for some time (Text-figs. 36 and 38). In other cases one of the synergids appears to be damaged by the entry of the pollen tube, while the other disorganizes later. During these events the filiform apparatus becomes less conspicuous, the striations appearing far apart and ultimately disappearing, while the basal granular part of the synergid remains visible for a considerable time as a highly staining mass. No male nuclei have been observed in the pollen tube during its growth, although they have been seen inside the sac lying near the egg-cell; in some instances one was found in contact with the egg-nucleus, while the other was on its way down the sac (Text-fig. 37). Though actual fusion has not been observed, several fusion nuclei with two distinct nucleoli have been found (Text-fig. 35). The union of the second male nucleus with the product of fusion of the polar nuclei appears to take place after fertilization.

The oval male nuclei follow the vegetative nucleus in the forward growth of the pollen tube of *Thesium* amid the glandular conducting tissue of the style. On reaching the ovary cavity it either grows along the surface of the placental column and the ovules or projects freely into the cavity until it reaches the upwardly directed apex of an ovule. The tube enters the ovule through the 'micropyle', and at the point of contact with the embryo-sac the membrane of the latter is dissolved. During the passage of the tube to the egg the synergids are not destroyed, but persist for a long time as disorganizing masses, staining

deeply with haematoxylin. Several instances of fertilized egg-nuclei with two nucleoli have been observed.

In *Osyris arborea* the course of the pollen tube is difficult to follow, although it is sometimes recognizable near the micropylar end of the embryo-sac. During penetration into the latter, one of the synergids appears to be partly destroyed. The male nucleus in contact with the egg-nucleus (Text-fig. 42), the fusion nucleus with two nucleoli, and the primary endosperm nucleus with three nucleoli have all been observed.

In *Scleropyrum* the path of the pollen tube in the stylar canal could not be traced. Evidence of fertilization is furnished by the presence of two nucleoli in the fusion nucleus (Text-figs. 44 and 45), and of triple fusion by the presence of two and three nucleoli in the nuclei of the endosperm cells. The role of the synergids in fertilization is similar to that in other Santalaceae.

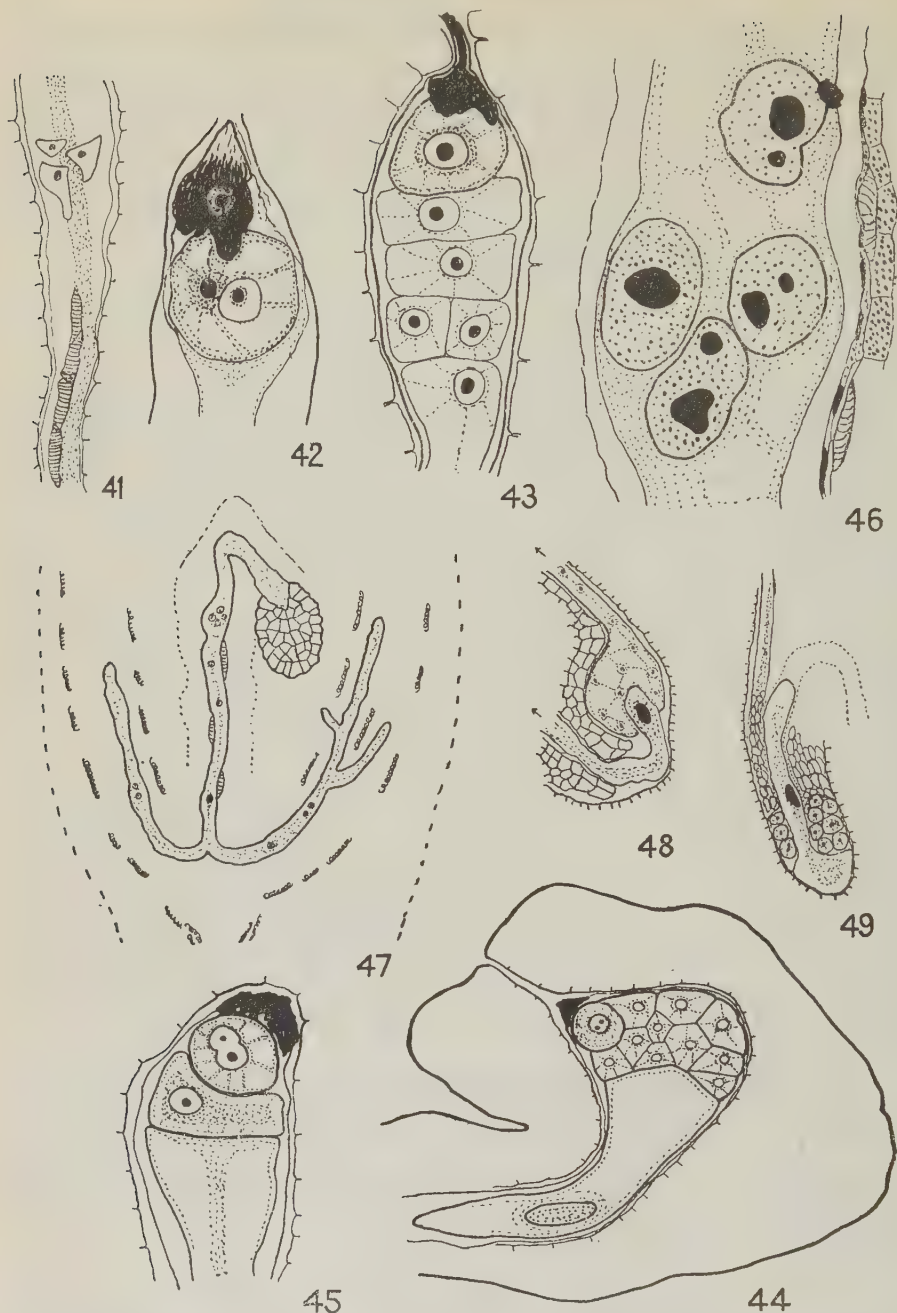
The detailed structure of the filiform apparatus of the synergids was studied in sections cut in different planes. In no instance could a system of tubular canals be recognized, such as is recorded by Schacht (1866) and later by Strasburger (1877), especially in *Santalum album*. In preparations stained with haematoxylin the body of the synergid is divisible into a lower half, which is usually densely granular and somewhat opaque with a prominent nucleus and vacuoles, and an upper half which is conical in shape and consists of a light-coloured matrix with groups of fine refractive striations, the filiform apparatus, converging to the apex of the cone. Transverse sections of the synergids present a corrugated contour, the striations appearing as thickenings of the plasma membrane, with thin intervening regions. There can be no doubt as to the glandular nature of the synergids and that it is their upper part that dissolves the apical part of the embryo-sac membrane. The latter is, however, still in contact with the synergids below the region of solution, which appears to be the line of demarcation between the upper and lower halves of the synergids. The equatorial indentations sometimes recognizable between the two halves seem to be due to the physical forces operating on the sac membrane and the synergids at the time of fixation. They do not appear to be permanent structures, since they are not found in all the synergids.

The visible changes in the embryo-sac after fertilization are (a) the growth of the chalazal end of the embryo-sac to form the antipodal haustorium, if this has not been previously produced; (b) the formation of endosperm; (c) the development of the embryo. Certain changes, closely associated with those within the sac, take place simultaneously in the ovary and assist in the supply of nutriment to the embryo.

#### FORMATION OF THE ANTIPODAL HAUSTORIUM

It is the antipodal end of the sac that grows into the placental column in Santalaceae and not a diverticulum of it as in *Fagus* or *Castanea* (Benson, 1894). In *Santalum album* the chalazal end of the sac, which was so far





TEXT-FIGS. 41-9. Fig. 41. Posterior end of the ovule of *Santalum*, showing the position and shape of the antipodals at the time of chalazal growth of the sac and the vascular elements which are partly destroyed by it. ( $\times 280$ .) Fig. 42. A stage in fertilization of *Osyris*. One of the synergids is disorganized, the other shows dense disintegration products. The male



confined to the ovule, resumes growth after fertilization. The greatly enlarged antipodal nuclei are left at the side, while the chalazal end of the sac penetrates the placental column, sometimes to its base. The digestive action of the growing haustorium is shown by the deeply stained disintegrated cells along its course. A similar digestive process plays a part in the growth of the micropylar haustorium. When all three sacs belonging to an ovary produce such haustorial outgrowths, the antipodal tubes may meet in the placental column and, perhaps as a result of pressure from the surrounding tissues, may fuse to form a single large tube (Text-fig. 51). The antipodal nuclei always remain in the part of the sac within the ovule and never pass into the haustorium. Though the micropylar haustoria are found growing side by side in the ovary cavity, they never exhibit fusion.

In *Osyris* and *Thesium* the growth of the antipodal end of the embryo-sac begins before fertilization, although it becomes much more rapid immediately afterwards. As in *Santalum* the haustoria of neighbouring sacs may fuse as they pass down the placenta. The haustoria may extend to the base of the placental column in *Thesium* and to the base of the strand of elongated cells in *Osyris* (Text-fig. 52). Disorganized cells occur all along the course of the growing haustorium, while absorption of nutritive material can be inferred by the presence of partly digested starch-grains in the placental cells adjacent to the haustorial tube. In the three genera under discussion the extreme ends of the tubes are blunt and show no thickening; this indicates that their function is not a mechanical penetration of the tissues. The haustorial tubes remain non-septate throughout and they do not become embedded in a vascular cup, formed by the carpellary bundles, as in *Myzodendron* (Johnson, 1889) and *Phoradendron* (York, 1913). In *Santalum* and *Thesium* the latter diverge far below the base of the placenta, while in *Osyris* the haustoria never grow beyond the base of the strand of elongated cells, which is far above the point of divergence of the carpellary strands.

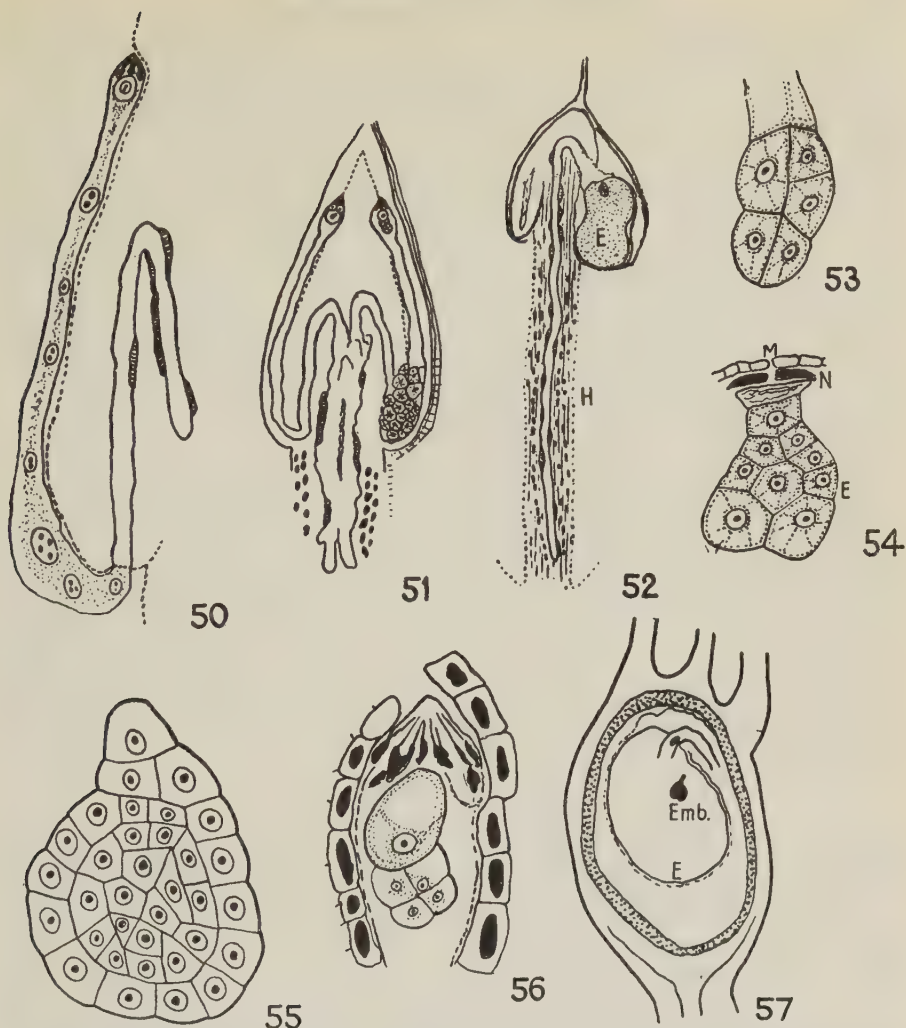
nucleus is in contact with the egg-nucleus. The cytoplasm of the egg-cell is highly vacuolated (side view). ( $\times 740$ .) Fig. 43. Embryo-sac of *Osyris* showing the fertilized undivided egg, remnants of the disorganized synergids and pollen tube, a number of endosperm cells, and the endosperm haustorial cell. ( $\times 500$ .) Figs. 44-7. *Scleropyrum Wallichianum*. Fig. 44. Portion of ovule in outline showing the fertilized and undivided egg, endosperm cells, and the endosperm haustorial cell with its large nucleus. ( $\times 230$ .) Fig. 45. An embryo-sac showing a stage in fertilization and endosperm formation. The endosperm cell has not yet divided. The endosperm haustorial cell has enlarged. Synergids are disintegrated. ( $\times 500$ .) Fig. 46. Portion of the antipodal haustorium of *Scleropyrum*, showing a group of antipodal nuclei which are very large, full of granular material and with a varying number of nucleolus-like bodies. The broken cells and disorganized vascular elements outside the haustorium can be seen. ( $\times 500$ .) Fig. 47. Portion of longitudinal section of an ovary showing the placenta whose boundary cannot be made out at this stage, one of the ovules with the developing endosperm, and its antipodal haustorium growing down the placenta and branching in the endocarp, with dispersed nuclei ( $\times 20$ .) Figs. 48 and 49. *Santalum album*. Fig. 48. Side view of the bend of the embryo-sac, showing several endosperm nuclei in the anterior limb and the endosperm haustorial cell entering the ovule posteriorly. ( $\times 65$ .) Fig. 49. The bend of the embryo-sac just outside the nucellus, showing the endosperm haustorial cell with its large nucleus on the left and endosperm cells on the right. ( $\times 65$ .)

*Scleropyrum* is unique as regards the behaviour of the antipodal haustorium which enters the placental column even before fertilization. The antipodal nuclei, moreover, migrate into the haustorium. After fertilization rapid growth of the haustorium ensues and simultaneously the antipodal nuclei enlarge greatly. During its penetration of the placental column the haustorium destroys the scattered vascular elements and, on reaching its base, it divides into a number of branches, which curve upwards and grow along the endocarp, branching repeatedly as they do so (Text-fig. 47). These ramifications of the haustorium usually follow the vascular strands in the ovary wall and some of them ultimately reach the base of the stylar canal. The antipodal haustorium thus forms a non-septate, highly branched tubular system, traversing the endocarp, which resembles the anterior end of the embryo-sac of *Dendrophthora* (York, 1913). The enlarged antipodal nuclei divide into a large number of nuclei of different sizes which occupy the haustorium and its branches. All the antipodal nuclei are several times larger than the original ones or those of the neighbouring endosperm cells. The hypertrophied condition of the nuclei and their distribution all over the tubular system are of great significance.

#### ENDOSPERM FORMATION

The formation of endosperm takes place in a slightly different way in *Santalum* from that in the other genera studied. The primary endosperm nucleus divides at the bend of the embryo-sac and a partition wall is formed between the two nuclei. The cytoplasm, surrounding the posterior nucleus, secretes at its surface a continuous membrane and constitutes a large cell occupying the entire sac. As a result of further elongation this cell assumes the shape of a cone, with the apex projecting into the antipodal haustorium; it constitutes the endosperm haustorium. The nucleus enlarges to several times its original size, but remains undivided up to the time of its final disintegration. The endosperm haustorium may elongate still further so as to reach the chalazal bend of the antipodal haustorium (Text-fig. 49). Simultaneously the anterior endosperm nucleus divides several times and some of the resulting nuclei migrate into the micropylar limb of the embryo-sac (Text-figs. 48 and 38). Wall-formation takes place later, and the endosperm tissue thus produced rapidly increases in amount so that the embryo-sac becomes distended and the surrounding tissues of ovules and ovary are pushed apart. The distended endosperm sometimes encroaches upon the endosperm haustorium and may surround it for some distance, thus giving, in longitudinal sections of the sac, the appearance of branches or diverticula (Text-fig. 48). The endosperm finally occupies the whole of the ovary cavity and surrounds the developing embryo, which lies near the base of the stylar canal.

In *Thesium*, *Osyris*, and *Scleropyrum* the primary endosperm nucleus divides into two nuclei around which two large cells, occupying the whole of the sac below the fertilized egg, are organized. The posterior cell elongates to



TEXT-FIGS. 50-7. Figs. 50 and 51. *Santalum album*. Fig. 50. Embryo-sac, with the fertilized egg and disorganized synergids at the anterior end, while in the cavity are several free nuclei of the endosperm. ( $\times 60$ .) Fig. 51. Extensive growth of the antipodal haustoria of two embryo-sacs into the placenta, where they have fused to form one tube. The egg-cells of the two sacs have been fertilized. ( $\times 45$ .) Fig. 52. Longitudinal section of a portion of a female flower of *Osyris*, showing the placenta, ovule, and the chalazal haustorium (H) extending into the placenta. Endosperm (E) has developed in one of the ovules. ( $\times 20$ .) Figs. 53 and 54. Stages in formation of embryo in *Thesium*. E = embryo, N = nucellus, M = micropyle. ( $\times 500$ .) Fig. 55. Stage in the development of the embryo in *Osyris arborea*. The large cell at the top of the pro-embryo represents the suspensor. ( $\times 500$ .) Fig. 56. Stages in the development of the embryo in *Santalum album*. The large suspensor cell disorganizes early. ( $\times 280$ .) Fig. 57. A longitudinal section of a fruit of *Thesium*. The thick dotted band represents the mesocarp which has become the stony layer. The embryo is deeply embedded in the endosperm tissue. ( $\times 20$ .)



form the conical endosperm haustorium, while the anterior one gives rise to the endosperm (Text-figs., *Osyris* 43 and *Scleropyrum* 44 and 45), which develops into a massive tissue occupying the ovary cavity and surrounding the embryo. The endosperm haustorium is much shorter than the antipodal haustorium of the sac and does not reach the base of the placenta, as it does in *Thesium montanum* (Schulle, 1933). The description and figures of the haustoria given by him and by Guignard (1885) make it evident that they did not clearly differentiate the two types of haustoria. According to Guignard the two nuclei, arising from the division of the secondary nucleus, are separated by a partition dividing the sac into an anterior half including the embryo and a posterior one containing a large nucleus, which does not divide further. The same view is expressed by later workers on Santalaceous plants, e.g. Modilewski (1928) and Iyengar (1937).

#### DEVELOPMENT OF THE EMBRYO

Early stages in embryo development have been studied in *Santalum*, *Thesium*, and *Osyris*. The fertilized egg only begins to divide when endosperm formation is far advanced. The first division of the egg is always at right angles to the long axis of the egg-apparatus. The upper cell or suspensor elongates somewhat but remains undivided for a long time, during which the lower segments by a vertical and a horizontal wall to form the four-celled embryo. In *Thesium* the suspensor elongates more than in the other genera, although it disappears early (Text-figs., *Santalum* 56, *Thesium* 53 and 54, and *Osyris* 55).

#### CHANGES IN THE OVARY

There are no seed-coats. Among the post-fertilization changes taking place in the ovary is the development of the endocarp, the cells of which increase in size and number and elongate, ultimately coming into contact with the cells of the placental column. After this the two tissues merge into one and completely fill the ovary cavity. At the outer limit of the endocarp there is a distinct meristematic zone, which contributes to the increase of the former. This is well marked in *Scleropyrum*, but only slightly indicated in *Santalum*, where the endocarp tissue does not completely fill the cavity of the ovary. In *Thesium* and *Osyris*, though some meristematic activity can be recognized, it does not contribute much towards the growth of the endocarp.

In the development of the fruit the middle layer of the ovary wall becomes stony, so that the ripe fruit is a nut, the stony layer of which cracks irregularly at the time of germination (Text-fig. 57). The radicle emerges from the massive endosperm and a thick collar is formed by the latter at its place of emergence.

#### DISCUSSION

In all four species the floral parts arise in acropetal succession and, after the carpels are formed, the floral axis develops into the placenta, from which three



ovules arise as lateral protusions. In all except *Osyris* the placenta is markedly lobed; in general shape it is conical. It becomes confluent with the inner wall of the ovary. The views held by Hofmeister (1859), van Tieghem (1869), and Warming (1878) regarding the nature of the placenta and the ovules in Loranthaceae, Santalaceae, and Balanophoraceae cannot be substantiated. Treub (1882) already pointed out that the 'mamelon' of Hofmeister is not an ovule reduced to its nucellus and that comparative study shows that it represents an outgrowth of the floral axis.

The ovules of Santalaceae have been described as naked, which is correct in so far as definite seed-coverings are not produced. The nucellus, in which the embryo-sac develops, is not, however, completely occupied by the latter. A considerable portion remains intact and functions as an integument. In *Thesium* and *Scleropyrum*, moreover, the cells at the apex of the nucellus divide so as completely to cover the sac, leaving a narrow passage which acts as a micropyle and which is used by the pollen tube in approaching the sac. Warming (1878) observed this in *Thesium ebracteatum*, where the nucellus itself gives rise to the integuments. Johnson (1889) in *Myzodendron punctulatum* records a single integument, which, as in *Osyris arborea*, is pushed apart by the growing micropylar end of the embryo-sac. Schulle (1933) in *Thesium montanum* points out that the deep-lying tissue plays a part in the formation of the thick integument.

All the floral organs are supplied by vascular strands from the bundles traversing the flower-stalk, while the placenta and its ovules receive no direct vascular tissue from these bundles. In *Scleropyrum* the carpellary strands divide into an inner and outer series, supplying different layers of the fruit-wall. The absence of a vascular supply to the placenta has been observed in *Myzodendron* by Johnson (1889). Guignard (1885) and Schaeppi and Steindl (1937) state that in *Osyris alba* the placenta receives a vascular trace derived from the branches of the carpellary strands; the diagram furnished by the latter (Fig. 1, *b*) is not convincing. In *O. arborea* no such vascular connexion to the placenta was found. The scattered vascular elements found in the latter and near the ovules in Santalaceae are altogether different from those in the vascular strands of carpels and perianth; the former are mostly short vessels with reticulate thickening, while the latter are elongated spiral vessels. The formation of isolated xylem elements in the nucellus has been observed also in other plants. Treub (1891) has described them in *Casuarina*, Benson (1894) in *Fagus* and *Castanea*, and Frye (1902) in *Asclepias cornuti*. In the absence of a clear demarcation between the ovule and its chalaza in Santalaceae, it must remain open whether the scattered vascular elements found near the embryo-sacs actually belong to the nucellus.

It has long been known that the embryo-sac of Santalaceae grows into the ovary cavity. Griffith (1845) was the first to describe this feature, though it appears to have been observed earlier. Guignard (1885) recorded elongation of the embryo-sac of *Osyris alba* at the summit until, as in *Thesium*, the

sexual apparatus was located close to the base of the style. Schacht (1866) demonstrated the growth of the sac into the ovary cavity and its branching in *Santalum album* and, like Griffith, described the division of the sac into a number of compartments near its lower bend. The formation of haustoria by the embryo-sac has been recorded also in other families, e.g. by Treub (1891) and Frye (1903) in *Casuarina*, by Benson (1894) in diverse *Amentiferae*, by Wylie and Yocom (1923) and Kausik (1938) in *Utricularia*. The nearest approach to the condition found in *Santalum* is that met with in *Amentiferae*.

The elongation of the embryo-sac in *Santalum album* into a micropylar haustorium, which carries the egg-apparatus to a position near the base of the stylar canal, may help in fertilization by bringing the egg-cell towards the pollen tube, a view propounded also by Benson (1894) for *Amentiferae*. In *Thesium* and *Scleropyrum*, on the other hand, the micropylar end of the sac remains within the ovule even after fertilization, although the opposite end elongates to form the antipodal haustorium. In *Osyris arborea* the small amount of growth at the micropylar end suffices to bring the sac to a position in which it just projects into the ovary cavity, while the chalazal end forms an antipodal haustorium. The embryo-sac is non-septate and unbranched (Schacht, 1866), being tubular throughout, although the diameter of the tube varies. Its wall comes into intimate contact with the synergids and, just before the entry of the pollen tube, that portion is dissolved, probably by a secretion produced by the synergids.

During fertilization in *Oenothera* (Ishikawa, 1918) the pollen tube, which extends to the synergids but never to the egg-cell, pierces the filiform apparatus; the synergids become much swollen with 'additional protoplasm' and the contents are suddenly chromatic. Later, the plasma membrane of the synergids bursts and the contents flow out upon the lower part of the oosphere. In *Santalum* instances of pollen tubes growing direct to the egg-cell, between the intact synergids, have been noticed, although usually one of the synergids is affected; in such cases the upper part of the synergids first undergoes disorganization, the lower part remaining visible for a long time. These facts indicate that the pollen tube is not responsible for the destruction of the synergids, but that they help in the entry of the former into the sac by a special secretion acting as a chemotropic stimulus. The increased secretory activity is indicated by the hypertrophy of the synergids and the accumulation of chromatic material. The secretion escapes through the apical portion, which usually becomes torn and leads to the destruction of the filiform apparatus. The cellulose reaction shown by the latter (Ishikawa, 1918; Habermann, 1906) is of interest. The function of the filiform apparatus is probably to protect the apex of the glandular synergid.

Endosperm formation has been described in several species of *Thesium* (Guignard, 1885; Modilewski, 1928; Schulle, 1933). In *Santalum album* it takes place by free nuclear division, but this is not so in the other three genera in which an endosperm haustorium is formed. There has been some un-

certainty as to the structure and position of the latter. According to Guignard (1885), after the division of the secondary nucleus of the embryo-sac in *Thesium*, a partition is immediately formed separating the sac into an anterior part which contains the embryo and becomes the seat of the endosperm and a posterior part containing the other daughter nucleus. The latter enlarges greatly, but does not divide further. This description, which has been followed by later investigators, does not explain the exact nature of the posterior compartment of the embryo-sac. Immediately after the formation of the partition a membrane is secreted at the surface of the cytoplasm in each part. This leads to the formation of an anterior endosperm cell and a posterior endosperm haustorial cell. In *Santalum album* only the posterior endosperm haustorial cell is produced, while the anterior part does not form such a cell, its nucleus later undergoing free nuclear division. However, the two compartments do not occupy the whole of the embryo-sac cavity, nor do they divide it into two. The endosperm cell divides to form a tissue, situated just behind the fertilized egg, while the haustorial cell elongates and becomes conical, with the apex projecting for some distance into the antipodal haustorium of the sac. The endosperm haustorium and the antipodal haustorium are therefore two separate entities, though the former develops inside the latter. This type of endosperm haustorium appears to be characteristic of Santalaceae, although a similar type is recorded in *Arum maculatum* by Jacobson-Paley (1920); here a row of five endosperm cells is formed within the sac, the lowest of which remains undivided and enlarges greatly to form the haustorium.

In *Myzodendron punctulatum* Johnson (1889) and Skottsberg (1914) describe a different method of endosperm formation. The secondary nucleus divides to form a long row of endosperm cells, which even extend into the antipodal haustorium. The cross-walls disappear from the part of the sac within the placenta and the liberated nuclei divide to form a long chain, for the most part embedded in dense granular cytoplasm. There is here no distinct endosperm haustorium. The free nuclei within the haustorium, in the placental region, perhaps have the function of facilitating the transference of nutritive materials to the growing embryo.

The part played by the antipodal cells appears to be insignificant. Modilewski (1928) is of the opinion that no tetrad is organized in the lower part of the sac of *Thesium intermedium* and that possibly only two nuclei are present, one of which functions as the polar nucleus while the other soon perishes. In the four species studied the antipodals vary in structure and function. In *Thesium* they are very small and seem to disappear immediately after fertilization, while in *Osyris* they become organized as regular cells and persist until the time of endosperm formation. In *Santalum* the antipodals, and especially the posterior one, enlarge at the time of fertilization, but they disappear when the endosperm haustorium extends into the antipodal end of the sac. In *Scleropyrum* the antipodal nuclei do not become organized as cells, although they show some degree of persistence. As the antipodal haustorium extends



into the placenta and branches within the endocarp, the antipodal nuclei appear to multiply and the products migrate into the haustorium and its branches. These nuclei are greatly enlarged and are rich in chromatin, characters which suggest that they play a part in the conduction of nutritive material to the growing embryo. Their hypertrophied condition indicating secretory activity (Tischler, 1921-2) renders it not improbable that they are also concerned in the transformation of nutritive material during its passage to the embryo.

The antipodals have long been regarded as rudimentary prothallial cells which have lost their original function and are now usually ephemeral. Westermaier (1890) first demonstrated the nutritive function of the antipodals, on the basis of their structure and micro-chemical reactions. His observations have been confirmed by Osterwalder (1898) and by Goldflus (1898-9), who based her conclusions on the anatomical structure of the tissue adjoining the antipodals in Compositae, as well as by Ikeda (1902) and by Lötscher (1905). The antipodal nuclei of *Scleropyrum* belong to the aggressive type of Coulter and Chamberlain (1904), in which active multiplication is associated with the penetration of the chalazal region by the antipodal portion of the sac; this type is characteristic of Rubiaceae (Lloyd, 1902), Compositae, Gramineae, and Amentiferae. In the typical example of *Aster Novae Angliae* Chamberlain (1895) reports a row of twenty antipodal cells extending into the basal part of the ovule as a haustorium, the lowest cell of the row becoming very large with dense cytoplasm and large nuclei. In *Grindelia squarrosa* Howe (1926) records that two of the antipodals persist without division, one or both of them forming haustoria, which penetrate the integument and extend nearly to the surface of the ovule. Campbell (1899) records other instances of antipodal growth in *Sparganium* and *Lysichiton*. He is of the opinion that the antipodal cells can not be regarded as mere vestiges of prothallial tissue, since their frequent physiological importance is amply evident.

#### SUMMARY

In four Indian species of Santalaceae, *Santalum album*, *Thesium Wightianum*, *Osyris arborea*, and *Scleropyrum Wallichianum*, the development of the floral parts, of the embryo-sac, of the endosperm and embryo, has been studied.

The placenta, about which there has been much controversy, is in all four species a continuation of the floral axis. It bears three pendulous ovules, which are naked except in *Thesium* and *Scleropyrum*, where the nucellus forms a single integument with a micropyle, facilitating the penetration of the pollen tube. The usually elongate placenta has no direct vascular supply from the main floral system. Some of the elongated cells in the axial region of the placenta are usually transformed into vascular elements which are isolated or in groups and may extend even to the ovules.



The embryo-sac, which is of the normal eight-nucleate type, shows a general tendency to grow out of the ovule. In *Santalum* it extends both in the anterior and posterior directions, forming haustoria. In *Osyris* the anterior growth is inconsiderable, while the posterior is extensive as in *Santalum*. *Thesium* and *Scleropyrum* show no anterior extension of the sac, but the posterior haustorium is extensive and, especially in *Scleropyrum*, ramifies within the endocarp.

Pollination and fertilization are normal. There is evidence of double fertilization in all four species. The synergids appear to have a glandular function, while that of the filiform apparatus is purely mechanical. The structure of the latter is described, and it is concluded that the striations are not constituted by canals or tubes.

The antipodals play an important part only in *Scleropyrum*, where they multiply and migrate into the antipodal haustorium of the sac and probably help in the transformation and translocation of nutritive materials to the embryo.

The mode of endosperm formation is, on the whole, uniform and distinctive of the family. In *Santalum* there is at first free-nuclear division, while in the others the endosperm is cellular from the beginning. In all four species an endosperm haustorium of characteristic shape is formed whose function is obscure.

The suspensor of the embryo disappears at an early stage.

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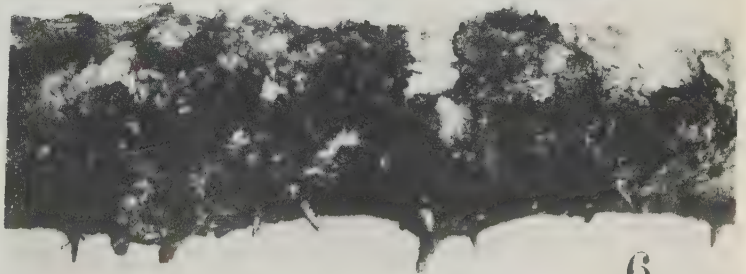
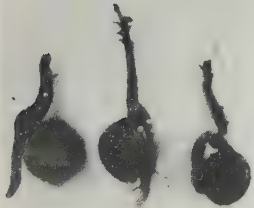
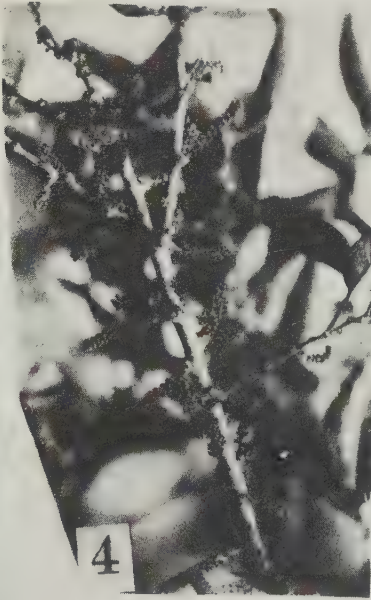
EXPLANATION OF PLATE IV

Illustrating the article by L. N. Rao on 'Studies in the Santalaceae'.

- Scleropyrum Wallichianum*. Fig. 1. Branch of a male plant, showing groups of catkins.  
Fig. 2. Branch from a female plant, showing three solitary spikes of female flowers.  
Fig. 3. Female spike.  
Fig. 4. Branch of male plant, showing catkins.  
Fig. 5. Twig with bunches of ripe fruits.  
Fig. 6. Portion of the stem, showing the spines.  
Fig. 7. Germination of seeds.







7

6



# A Technique for the Anatomical Study of Root Parasitism

BY

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With Plate V

STEPHENS (1912) was the first to study the structure and development of the haustoria of *Striga lutea* Lour. parasitic on maize roots. Later, Saunders (1933) carried out more extensive studies on the parasitism of this plant and found certain differences between resistant and susceptible strains of *Sorghum vulgare* in regard to the rapidity and extent of penetration of the parasite.

In our investigations on the attack of *Striga*, a root parasite on *Sorghum vulgare* and on several other hosts (Kumar and Solomon, 1941), we have examined three species of the parasite, viz. *Striga lutea*, *S. densiflora*, and *S. euphrasioides*. Any attempt to study by the usual methods of section cutting the differences in the type of attack by different species of *Striga* on different varieties of *Sorghum* possessing varying degrees of resistance to parasitism would be most laborious and time-consuming. It was therefore found necessary to devise a technique by which the whole material is made nearly transparent and then any desired tissue, in the present case lignified tissues, stained with an almost transparent stain. The method had to be rapid and easy of manipulation in order to deal with the large amount of material under study.

Various techniques and their modifications were tried but the results were not satisfactory. The method suggested by Debenham (1939) for the microscopic examination of the xylem of whole plant organs, besides taking too long, did not give very transparent preparations. After several trials it was found that staining by the Feulgen technique after properly clearing the material gave very satisfactory results. Subsequently, to test the possibility of wider application of this method, the technique was tried on a variety of other materials including fern fronds, *Selaginella* stem, roots of seedlings, flower buds, leaves, &c. The results showed that if the material is not very thick and can be well cleared, the lignified elements take on a very characteristic, bright, reddish-violet colour.

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## MATERIAL

Striga seeds were sown in a Petri dish with moist blotting-paper at the bottom and one or two grains of Sorghum were placed in the centre and surrounded by a wad of cotton to keep them moist. The Petri dish is covered with a piece of cardboard, slit on one side to allow the Sorghum leaves to come up, and the dish is kept in an incubator at 30° C. in the dark. Within two to three days the Sorghum grains germinate and the main root grows horizontally on the blotting-paper, which is kept moist by the addition of a little water every day. The main root gives rise to many lateral roots. The Striga seeds which lie near the host roots germinate and establish contact with them. The dishes are examined every day and material collected for fixation. It was found possible to procure enough material in the different stages in about two weeks.

## FIXATION

As materials are to be mounted whole, they should be of convenient size and not larger than is necessary for the particular study. Small lengths, about 0.5 to 1 cm., of the host root with the parasite attached are cut with a pair of sharp scissors and immediately transferred to the fixative. Hillary (1939) has shown that for successful application of the Feulgen technique a fixative containing chromic acid should be used. We have used, with uniformly good results, Nawaschin's fluid both for cytological and morphological studies, and as materials may be left in this fixative for a week or two without any damage, it was used throughout the investigation.

## WASHING AND CLEARING

After keeping the material in the fixative for at least 24 hours it is washed in running water for one hour and then passed to 70 per cent. alcohol after keeping in 30 per cent. and 50 per cent. alcohol for one hour each. The material is left in 70 per cent. alcohol at least overnight; this hardens the material and prevents maceration during subsequent treatment. Material which cannot be handled immediately may be stored in 70 per cent. alcohol.

The fixed material is brought down to water after passing through 50 per cent. and 30 per cent. alcohol and given a few changes of water. The method then used is that devised by Schultz (1897) for demonstration of bone in mammalian embryo in which the soft tissues are cleared with potassium hydroxide. The material is left in 1 per cent. potassium hydroxide solution for one to two days. A higher concentration of hydroxide tends to macerate soft tissues. When the material is properly cleared the vascular strands should be easily visible through the outer tissues, which should be almost transparent. The cleared material is then washed in running water for about 30 minutes and is ready for staining.



## STAINING

This is done by a modification of the Feulgen reaction now widely used for chromosome studies (de Tomasi, 1936). The Feulgen reaction as applied to chromatin involves a chemical reaction in which as a result of a mild hydrolysis of thymonucleic acid contained in the chromosomes an aldehyde is liberated. This aldehyde when brought into contact with decolorized fuchsin sulphurous acid restores the natural colour of fuchsin and thus stains the chromosomes containing the liberated aldehydes. Margolena (1932) has stated that lignin, suberin, and cutin also give a persistent positive reaction with the Feulgen test. Relying on the observations of Mehta (1925), she infers that this positive reaction is due to the presence of aldehydes in the molecules of these three substances. In the present technique this positive reaction of lignin to the Feulgen test is applied in staining the lignified elements of both host and parasite. The most important points on which a successful reaction depends are (1) use of an absolutely colourless 'leuco basic fuchsin', and (2) correct hydrolysis.

Basic fuchsin suitable for this purpose is difficult to obtain. We tried four samples of basic fuchsin, and of these Grubler's special basic fuchsin gave the best result, being superior to the certified stain supplied by Coleman & Bells (certificate No. CF. 17). The stain is prepared in the following way: pour 100 c.c. of boiling distilled water over 0.5 gm. of powdered basic fuchsin in a flask; shake vigorously and allow to cool to 50° C.; filter into a bottle with a ground glass stopper and add 10 c.c. of N/HCl; cool to 25° C. and add 0.5 gm. of potassium metabisulphite. Leave the bottle always well stoppered and in a dark place. The liquid becomes colourless within 24 hours and can be used till it begins to assume a reddish tint due to oxidation.

As shown by Hillary (1939), hydrolysis is a critical part of the procedure and the optimum period of hydrolysis varies with the fixative used and sometimes also with the material. With Nawaschin's fixative, 15 to 20 minutes hydrolysis in dilute HCl (8 c.c. of concentrated HCl of s.g. 1.19 to 100 c.c. of distilled water) at a constant temperature of 60° C. in an electric incubator was found the best for the materials used in the present study. Material which is ready for staining is transferred from water to cold HCl of the same strength as used for hydrolysis. After 1 or 2 minutes it is transferred to dilute HCl at 60° C. and hydrolysed for 15 mins. Then the acid is poured off and some decolorized fuchsin solution, prepared as detailed above, is added. The material is left in this solution from 6 hours to overnight. Then it is washed in running tap-water for about 10 minutes and then given two or three changes of water made slightly alkaline by the addition of a few drops of 1 per cent. KOH solution; this treatment intensifies the stain. It is found advantageous to omit the usual washing in SO<sub>2</sub> water, as the retention of a very light stain in the cellulose walls is found desirable. The material is then rinsed in distilled water and passed up rapidly through alcohol grades of 30, 50, 70, and 95 per

cent. strength, allowing about 3 minutes in each. Then it is changed to absolute alcohol, then to xylol-alcohol, keeping in each for about 5 minutes and finally taken to pure xylol. After leaving in xylol for 1 hour the material is mounted in thin canada balsam. The slide is placed horizontally on a hot plate at about 40° C. for a few hours with a 5 gm. weight on the coverslip. This helps to flatten slightly the material and is useful for certain types of observations. More balsam is added at the edge of the coverslip if necessary.

#### COMMENTS

Previous studies on the structure and development of haustoria of *Striga* give only details of certain stages, as they have been mainly based on serial sections (cf. illustrations of Stephens (1912) and Saunders (1933)). The advantages of the present technique are: (1) it is possible to follow all the stages from germination of the *Striga* seed to the development of the adult parasite; (2) a complete and clear idea of the type and degree of haustorial penetration at any stage of development of the parasite on the host root can be obtained; and (3) it is easy to make comparative studies under controlled conditions. However, it is not to be expected that this technique, though facilitating anatomical examination of unsectioned material, will completely eliminate the need for section cutting; it reduces considerably, however, the latter type of work and is distinctly superior to it for the type of observations mentioned above. Another advantage is that material which has been stained and brought to the xylol stage can be examined under the microscope, rough diagrams made of the haustoria, and then, if desired, can be taken to paraffin for embedding in the usual manner and serial sections made which can be counterstained with light green or any other suitable stain and examined. These observations together with those made prior to embedding the material give a much clearer idea of the anatomy than can be obtained from serial sections alone.

It is hoped that this technique, with modifications where necessary, will prove to be of wider application. Plate V shows photomicrographs of preparations made by this method. Brief notes on the anatomical features are given in the explanation to the plate. A detailed anatomical account will be published when the studies now in progress are completed.

#### SUMMARY

A new technique for the anatomical study of root parasitism is described. It involves fixation of the material in Nawaschin's fluid, clearing in 1 per cent. potassium hydroxide solution and then staining by a modification of the Feulgen technique.

The advantages of this technique over the usual method of sectioning are indicated.

This research was carried out under the 'Scheme of Investigations into the

attack of *Striga* on *Sorghum*', financed by the Imperial Council of Agricultural Research, New Delhi.

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### EXPLANATION OF PLATE V

Illustrating the article by L. S. S. Kumar, A. Abraham, and S. Solomon, on 'A Technique for the Anatomical Study of Root Parasitism'.

The photomicrographs are mounts of portions of *Sorghum* root with *Striga* seedlings in various stages of parasitic development upon it. Figs. 1-6 and 10 are of *Striga lutea* while Figs. 7-9 are of *S. densiflora*.

Fig. 1. *Striga lutea*. The seed has germinated and the end of the radicle which forms the primary haustorium has penetrated the cortex of the host root. The cotyledons are still enclosed by the seed coat. Some idea of the transparency of the preparation can be obtained from the fact that though the *Striga* radicle passes below the *Sorghum* root it is very clearly visible. ( $\times 40$ .)

Fig. 2. The left-hand portion seen in the above figure under higher magnification. Under this focus it would appear that the haustorium is not in contact with the vascular cylinder of the host, whereas in another focus (see Fig. 1) it is clearly seen to be in contact with it. ( $\times 130$ .)

Fig. 3. The haustorium has enlarged; vascular elements are developed in the parasite and close contact with the vascular elements of the host is established, though no penetration into it has taken place. The origin of a branch, which later develops into a secondary haustorium, is seen as a lateral outgrowth to one side of the primary haustorium. ( $\times 80$ .)

Fig. 4. A later stage after the cotyledons and plumule have come out of the seed coat. Haustorial cells have penetrated into the vascular tissue of the host. The first pair of leaves are seen as rudiments. ( $\times 80$ .)

Fig. 5. Still later stage showing deeper penetration of the host tissue by the haustorial cells. ( $\times 80$ .)

Fig. 6. Portion of the above under higher magnification to show more clearly the haustorial connexion. ( $\times 160$ .)

Fig. 7. *Striga densiflora*. A portion of the host root attacked by two *Striga* seedlings from opposite sides showing that penetration of a seedling by one haustorium does not interfere with the penetration by another. The seedling on the upper side is apparently older than the one below, in which the cotyledons are still enclosed by the seed coat. ( $\times 50$ .)

Fig. 8. The haustorial connexion of the above under higher magnification, showing clearly the tracheides of the parasite and 'haustorial hyphae', which are connected to the tracheides and have deeply penetrated the host vascular tissue. ( $\times 160$ .)

Fig. 9. Another specimen of *S. densiflora* showing the very deep penetration by the haustorial cells; note the swollen and branched ends of these cells. From comparison of this with *S. lutea* of the same stage (see Fig. 10) it is seen that *S. densiflora* shows much deeper penetration of the vascular tissue; a fact which accounts for the observation that the latter is more injurious than *S. lutea* in its attack on the host. ( $\times 160$ .)

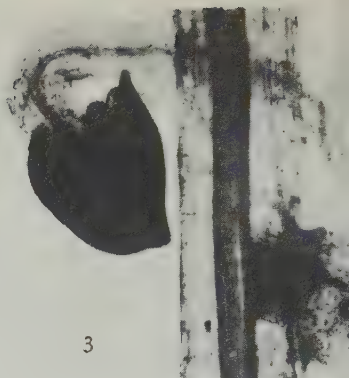
Fig. 10. *S. lutea*. Haustorial region magnified, showing clearly the lignification of the xylem elements of both host and parasite. A comparison with Fig. 9 shows that the penetration in this case extends only as far as the centre of the vascular cylinder of the host. ( $\times 160$ ).



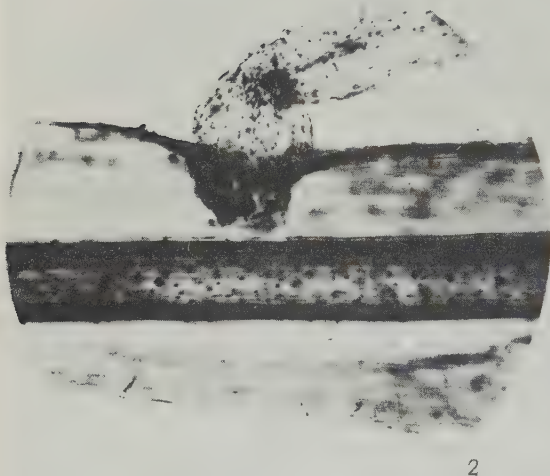




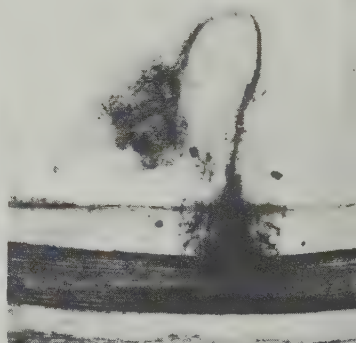
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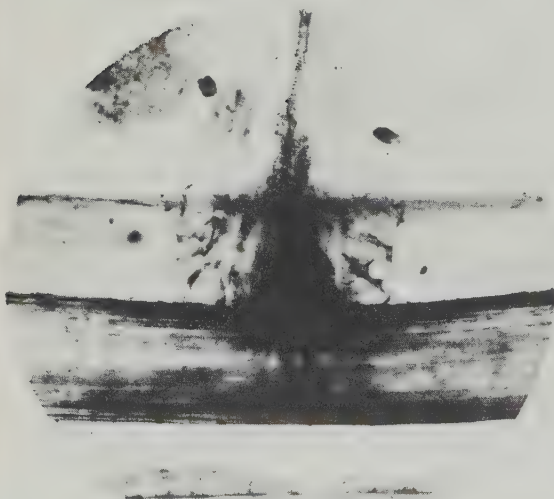
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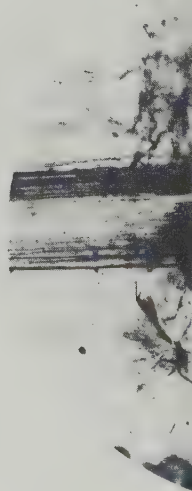
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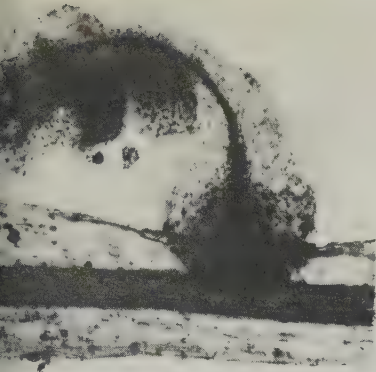


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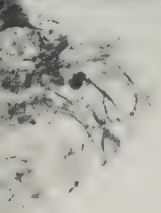
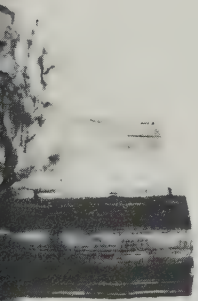




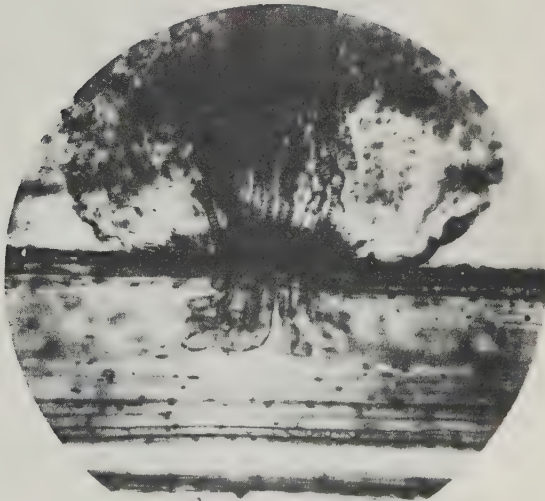
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# Chromosome Studies in some British Species of *Limonium*<sup>1</sup>

BY

H. C. CHOUDHURI

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With one hundred and fifteen Figures in the Text

THE occurrence in nature of interspecific hybrids among the British species of *Limonium* has been suspected since 1902, when Linton and Marshall discovered *L. Neumani* at Bosham; Neuman had previously reported this plant from Denmark, as a hybrid between *L. vulgare* and *L. humile*. De Fraine and Salisbury (1916) recorded a broad-leaved form of the variable *L. binervosum* from Blakeney Point, Norfolk; this plant, which shows certain characters intermediate between *L. binervosum* and *L. bellidifolium*, occurs only in habitats occupied by both species, and they suggested the possibility that it was a hybrid between the two species.

The present study was instituted primarily to ascertain if cytological evidence could be obtained to confirm the views of the taxonomists mentioned above in regard to *L. Neumani* and the broad-leaved form of *L. binervosum*.

The following species and varieties were studied:

*L. bellidifolium* Dum. = *Statice reticulata* Sm.

*L. binervosum* Salmon = *Statice binervosa* G. E. Smith.

*L. binervosum* Salmon, Broad-leaved forms.

*L. vulgare* Mill. = *Statice limonium* L. pro parte.

*L. rariflora* O. Kuntze = *L. humile* Mill.

*L. Neumani* Salmon = *L. rariflora* × *L. vulgare*.

## METHODS

All these plants are found at Blakeney Point, Norfolk, where most of the material was fixed in the summer of 1938. Some was also obtained from plants from the same locality, grown at University College, London.

For examination of the somatic chromosomes, root-tips fixed in weak Benda's fluid, or in Flemming's strong mixture diluted with an equal volume of water, proved satisfactory for the study of chromosome morphology. Navashin's chrom-acetic-formalin mixture, while satisfactory for chromosome counts, tended to produce swelling of the chromosomes.

For the meiotic divisions pollen mother-cells were studied. Meiosis takes

<sup>1</sup> Part of a thesis approved for the degree of Doctor of Philosophy in the University of London.



place when the buds are very small. In general Kihara's method was adopted, the buds being placed for 1 to 2 minutes in Carnoy's fluid (6 : 3 : 1), and thence into a definitive fixative, of which Flemming's strong fluid proved most useful. Carnoy's fixative used alone gave satisfactory fixation for certain stages.

Material was stained by Newton's Gentian Violet, Iodine technique. With material fixed in Carnoy's fluid alone it was necessary to treat sections with 1 per cent. chromic acid after staining, to prevent too rapid removal of the stain; the same solution was sometimes used as a mordant.

### *Chromosome Number and Morphology.*

In the present investigation little attention has been given to chromosome morphology, but the chromosome complements of the species (*L. bellidifolium*, *L. binervosum*, *L. vulgare*, and *L. rariflora*) are figured (Figs. 1-9); these figures were made from root-tip cells.

In *L. bellidifolium* (Figs. 1 and 2) there are 18 chromosomes, of which all but one pair have submedian attachment constrictions; in the remaining pair the attachment constriction is median.

In *L. binervosum*, which has long attracted attention by its 'puzzling variability' (Salmon, 1907), only plants which were regarded as typical were studied; these possessed 32 chromosomes (Figs. 3 and 4), which may be arranged approximately in 4 sets of 8; in each set one chromosome has a submedian attachment constriction, the remainder submedian or subterminal ones.

*L. vulgare* has 32 as its somatic number (Figs. 5 and 6), and here again there are 4 sets of 8 chromosomes, all with subterminal or submedian constrictions.

In the 36 chromosomes of *L. rariflora* nine different types may be distinguished (Figs. 7 and 8), and of these nine types one has a median attachment constriction and eight submedian ones.

Thus of the four species examined *L. bellidifolium* is a diploid species with 9 as its basic number, *L. rariflora*, with the same basic number, is a tetraploid, while *L. binervosum* and *L. vulgare* are tetraploids with a basic number of 8.

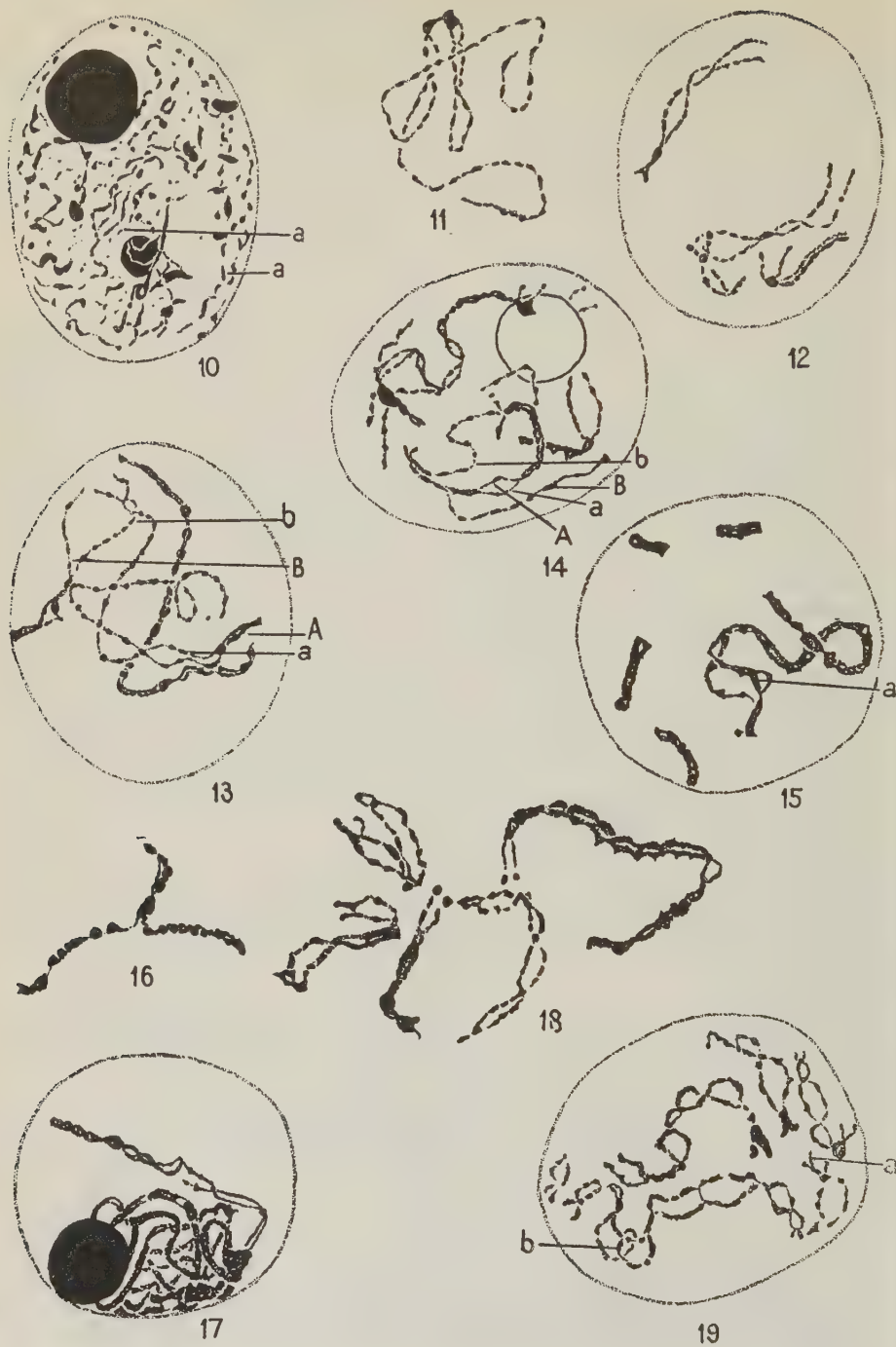
### *Nucleolar Bodies.*

In the nucleolus, at mitosis, one or more deeply stained bodies of a crystalline appearance are present (Fig. 9 and 9a). These bodies may be differen-

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All drawings were made with the aid of a camera lucida, using a Beck 2 mm. 1.4 N.A. apochromatic objective. Letters at end of each legend denote fixative used, viz. BE., weak Benda's fluid; FL., Flemming's fluid, half-strength; CA., Carnoy's fluid. Where no fixative is noted Flemming's fluid, full strength, was used.

FIGS. 1-9a. 1. *L. bellidifolium*, metaphase plate from root-tip; BE. 2. Idiogram of 1. 3. *L. binervosum*, metaphase plate from root-tip; FL. 3a. Anaphase chromosomes from somatic cell, showing submedian attachment region; FL. 4. Idiogram of 3. 5. *L. vulgare*, metaphase plate from root-tip; BE. 6. Idiogram of 5. 7. *L. rariflora*, metaphase plate from root-tip; FL. 8. Idiogram of 7. 9. *L. rariflora*, nucleolus from root-tip cell, with numerous deeply stained bodies of more or less crystalline form. 9a. As 9, but showing the differentiation of the deeply stained bodies into inner and outer regions. All  $\times c. 3,060$ .





tiated into inner and peripheral regions (Fig. 9a), owing, it is considered, to the appearance of a central vacuole, which presumably owes its formation to the withdrawal of material from the centre of the body, or to some alteration of the material in this region.

Nucleolar inclusions, variously referred to as crystalline and proteinaceous, have been recorded previously from several plants (cf. Reed, 1914). It would seem, therefore, that these bodies are not always of the same nature, and it may be surmised that they are connected in some way with the metabolic processes of the cell.

#### MEIOSIS: GENERAL DESCRIPTION

The earlier meiotic stages do not fix well, hence material for critical study was limited. The most favourable material for the study of chromosome behaviour in these early stages was that provided by *L. rariflora* and *L. vulgare*, although the other species, as well as the hybrids, were also examined.

Prophase of the meiotic division begins with the appearance of slender, somewhat twisted, threads, which cannot be followed continuously for any considerable length; the occasional free ends indicate that there is no continuous spireme. The structure of these threads could not be ascertained. At this time some of the threads can be seen lying parallel with others through part of their length, although they do not actually come into contact with one another (Fig. 10, a). Later the beaded appearance of these chromosome threads becomes marked, and the chromomeres vary in size and spacing (Fig. 13).

Chromosome pairing begins at a point and passes along the length of the threads; hence at an early stage of pairing the threads lie parallel along part of their length, while elsewhere and at their ends they lie free, and sometimes widely separated (Figs. 11, 12). The points at which pairing begins are more deeply stained than other parts of the chromosomes and are probably the centromere regions, which are more contracted than other parts of the chromosomes. Darlington (1937) notes that the chromosomes in *Fritillaria* are more deeply stained in the centromere region, and that it is near here where pairing probably begins.

FIGS. 10-19. 10-16. *L. rariflora*. 10. Late leptotene; in places (a), the threads are beginning to pair. 11. A bivalent at early zygotene, the ends lying free and widely separated. 12. Three bivalents at zygotene; pairing in the lowermost is nearly complete, in the others only short lengths are paired. 13. Zygotene; pairing between four chromosomes (A, a, B, b): B, b, incomplete. 14. Zygotene; in the lowermost, quadrivalent association, pairing between the threads A, a, over a long distance appears to have prevented the pairing of the threads B, b, except at one end: one end of the uppermost bivalent is in contact with the nucleolus and is marked by a deeply stained region. 15. Late pachytene; in the long bivalent the unpaired region shows chromatids, the formation of which has probably anticipated pairing in this region; CA. 16. A tetravalent at pachytene, the bivalents associated only over a short distance. 17. *L. vulgare*, Synzeosis: the emerging thread shows clearly its double nature; CA. 18. *L. rariflora*, diplotene bivalents. 19. *L. rariflora*, diplotene bivalents, a later stage than 18; note doubleness at one end of a chromosome (a), and inversion loop (b); CA.

In the tetraploid *L. rariflora* pairing takes place at random between four threads, which meet only in pairs (Fig. 13, *A, a, B, b*), although any one of the four is not necessarily paired with any other one throughout its length. Pairing would seem to be initiated at a point, and to pass along the length of the chromosomes until interrupted. Further, where two chromosomes which lie between the other two homologous chromosomes are paired for a long distance, pairing of the two outer ones is interfered with; cf. Fig. 14.

Pairing is often incomplete at the ends of the chromosomes remote from the point at which it begins. In particular, it is interfered with in the region where the chromosome is attached to the nucleolus (Fig. 14): it may be surmised that pairing will be delayed in chromosome segments attached to the nucleolus, owing to the difficulty in movement of the homologous threads (Darlington, 1935).

In the long chromosomes the ends often remain unpaired, presumably because the time available for pairing is short and the ends lie farthest from the point at which pairing begins. In Fig. 15 the unpaired distal segments of a long bivalent show splitting in one of the chromosomes (*a*), even though the threads in the paired region are still unsplit. It thus appears that when pairing is delayed it may never be completed, as division of the homologous threads interrupts pairing, as suggested by Darlington (1937).

When pairing ends the chromosomes become thicker and shorter, and at this time seem to contract slightly to one side of the nucleus. Tight synezetic knots were sometimes observed, but these are regarded as artifacts, produced by fixation, accentuating the real contraction of the chromosomes; at this period the nucleus seems to be specially intolerant to fixatives, and contraction is thus often exaggerated; in the apparently exaggerated contraction shown in Fig. 17 the double nature of the chromosome threads is well shown.

Diplotene begins with the separation of the paired chromosomes (Fig. 18), but the double nature of the individual chromosomes is rarely visible except at the ends (Fig. 19, *a*). The opening out of the paired threads in any nucleus is not simultaneous, and bivalents with widely separated chromosomes may be found side by side with others in which little separation has occurred (Fig. 18); chiasmata appear as the chromosomes open out. In the bivalents the chromosomes are often free at the ends (Fig. 19), the chiasmata being localized.

By mid-diakinesis the doubleness of the individual chromosomes is more clearly visible (Fig. 21). Further shortening of the chromosomes, accompanied by their greater separation, follows, until by late diakinesis they remain associated only by one or two chiasmata. At this stage the bivalents are scattered throughout the nucleus, owing, presumably, to strong repulsion between the chromosomes. Short chromosomes are mostly held by a single terminal chiasma.

Mention may be made of the behaviour of the nucleolus during meiosis. Certain of the zygotene bivalents lie in contact with the nucleolus, the point of contact being marked by a deeply stained region (Fig. 14), and at diakinesis

certain of the bivalents often lie on the nucleolus, although it has not been ascertained if the nucleolus is formed from any organized region of the chromosomes; that this is possible, however, is suggested by the frequent proximity of nucleolus and bivalents. Contact between nucleolus and chromosomes has been demonstrated for other plants (cf. Parthasarathy, 1939; Bhaduri, 1940).

In the nucleoli during meiosis deeply stained bodies of a crystalline form, similar to those noted by numerous authors (cf. Latter, 1926), were sometimes observed (Fig. 20, a).

### *Cytomyxis.*

The passage of some chromatin from a pollen mother-cell into an adjacent one (cytomyxis) has frequently been recorded (cf. Church, 1929). This phenomenon has been noted only at synezeisis, a stage at which the nucleus is specially sensitive to the effects of fixatives, and this has led to the opinion, generally held, that the process does not occur naturally, but only as a direct result of fixation or by reason of some abnormal condition which has led to the breakdown of the cell wall and nuclear membrane. In Fig. 22, however, where an example of cytomyxis is figured, it will be observed that there is no indication of marked contraction of the nuclei, such as might be associated with faulty fixation; this example may possibly indicate that this peculiar phenomenon is real, and that it may take place in the early stages of pollen development; nevertheless, it must be emphasized that most of the examples of cytomyxis which were encountered were undoubtedly exaggerated by fixation, if not the direct result of this process.

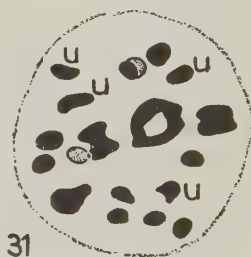
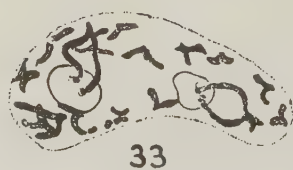
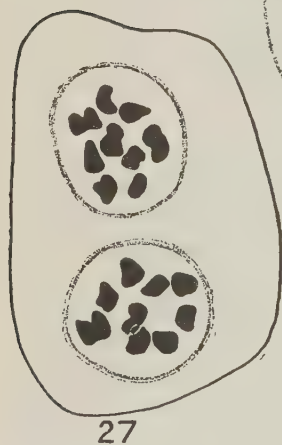
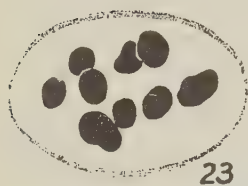
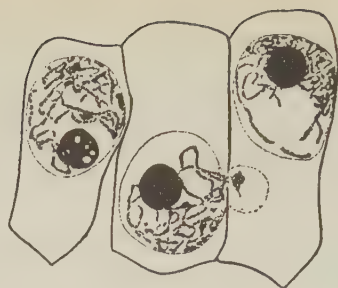
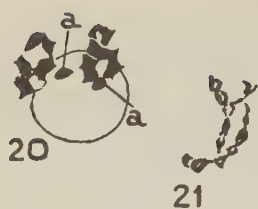
### *The Tapetum.*

The not unusual feature of bi- or multinucleate tapetal cells is well shown in *Limonium*, in which, in the species under consideration, the cells are usually binucleate by pachytene, although broader and shorter uninucleate cells may also be seen at this stage, and less frequently cells with four nuclei.

The tapetal cells frequently project into and reach across the pollen sac, which is thus cut into several compartments; in such a compartment there may be no more than a single pollen mother-cell; such isolated pollen mother cells were usually found at the ends of anthers, although they sometimes occupied a more median position. A similar arrangement of the tapetum has been noted by Clausen (1926) in *Viola* hybrids, and by Meurman (1928) in *Ribes Gordonianum*; it no doubt results in a more efficient nutrition of the pollen mother-cells.

During later stages of meiosis the tapetal cells undergo great elongation in a direction parallel to the long axis of the anther, and by the time the pollen lies free in the pollen sac the tapetum remains as a thin layer.

Normally the pollen mother-cells show a uniseriate arrangement, but sometimes two cells lie side by side. The origin of these more laterally placed cells





is uncertain; they may represent actual pollen mother-cells or transformed tapetal cells; they are at the same stages of division as the other pollen mother-cells, and their nuclei behave normally during the early meiotic stages; later the cells apparently disappear, but it is uncertain whether they, like the tapetal cells, are used for the nutrition of the developing pollen mother-cells, or if they become pressed into line with the elongation of the anther.

### *Meiosis in the Species of Limonium.*

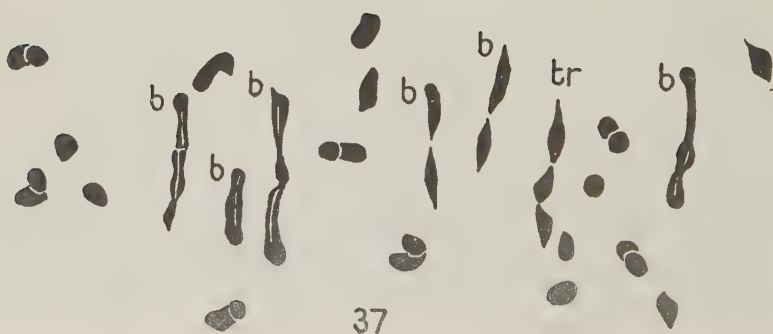
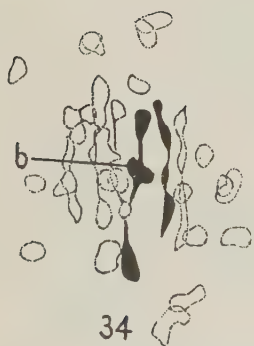
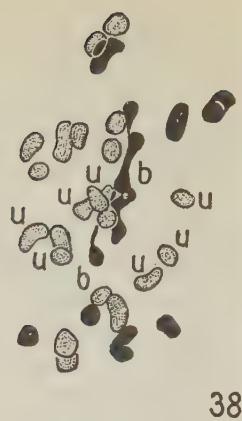
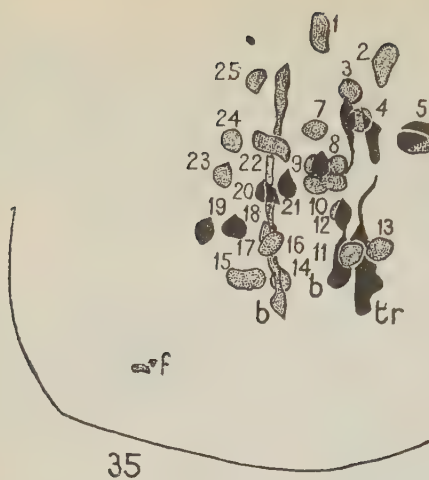
(a) *L. bellidifolium*. In this species meiosis is regular (Figs. 23–8), and the nine bivalents become evenly orientated on the equatorial plate (Figs. 23, 24). First anaphase figures were rarely seen, and it is therefore inferred that this stage is of short duration, although interphase, to judge from the frequent occurrence of interphase nuclei, is a lengthy stage. Meiosis is not simultaneous in all the cells of an anther, and first and second metaphase figures with intervening stages may be found in a single pollen sac.

The chromosomes are visible at interkinesis (Figs. 25, 26), when the haploid number is easily observed; sister chromosomes lie side by side, held together by their attachment regions. Attention may be drawn to a singular pair figured (Fig. 25, *A, a*) in which the attachment region has divided precociously, and repulsion between the two chromosomes exists throughout their length (cf. Darlington, 1937).

During interkinesis nucleoli appear as small bodies which may be attached, or at least closely apposed, to the chromosomes (Fig. 26). These small nucleoli apparently arise *de novo*—at least, there is no indication of their survival from earlier stages; subsequently they coalesce to form a larger one. Kulkarni (1929) has recorded the presence of similar nucleolar bodies in interkinesis in *Oenothera pratincola*.

From interphase onwards deeply stained granules of varying size often appear in the cytoplasm (Figs. 26, 28). Lack of material precluded the application of adequate chromatin tests on these granules, but it seems probable that they are nucleolar material which has been extruded from the nucleus at interkinesis, or possibly earlier.

FIGS. 20–33. 20. *L. binervosum*, diakinesis, showing two bivalents lying on nucleolus; nucleolus contains bodies of crystalline form (*a*); CA. 21. Broad-leaved *L. binervosum*, a bivalent at mid-diakinesis, showing doubleness of the individual chromosomes; sister chromatids are twisted round one another except in the region of the chiasma. 22. *L. vulgare*, three pollen mother-cells, the middle one showing cytomyxis; CA ( $\times 875$ ). 23–8. *L. bellidifolium*. 23. First metaphase with 9 bivalents. 24. First metaphase complement, lateral view. 25. Interkinesis: the large pair of chromosomes (*A, a*) have separated; their attachment regions lie at opposite ends. 26. Interkinesis: minute nucleolar bodies are attached to some of the chromosomes; there are two deeply-stained bodies in the cytoplasm. 27. Second metaphase, with 9 chromosomes on each plate. 28. Second telophase with 9 chromosomes at each pole; there are two granules in the cytoplasm. 29–33. *L. binervosum*/39. 29. Diakinesis, with 16 bivalents; CA. 30. First metaphase with 16 bivalents; CA. 31. First metaphase showing 18 chromosomes; presumably 14 bivalents and 4 univalents; CA. 32. A very regular first metaphase with 16 bivalents, lateral view; CA. 33. Interkinesis, chromosomes held together only at attachment regions; ends of the two long pairs lie on the nucleoli; CA.



(b) *L. binervosum*. Fixation of the earlier meiotic stages was generally poor, although a few diakinetik nuclei were well fixed. At diakinesis 16 bivalents are distributed more or less equidistantly through the nucleus. In the smaller bivalents the chromosomes lie side by side, or are sometimes held by terminal associations, while in the larger bivalents the chiasmata are often at either end, so that ring bivalents are produced (Fig. 29).

At first metaphase there are 16 bivalents, of which one is usually of the ring type (Fig. 30) and often breaks at this time. One metaphase plate showed the unusual feature of 14 bivalents and 4 univalents (Fig. 31).

As might be expected in so variable a species as *L. binervosum*, all stages between regular and highly irregular metaphases were observed. Thus *binervosum*/39 showed fairly regular metaphases (Fig. 32), with 16 bivalents arranged at the equator; *binervosum*/3, however, showed irregular metaphases with a high frequency of univalents, which generally failed to take their place with the bivalents in the equatorial region. Thus Fig. 36, taken from a cell of this plant, shows a lateral view of first metaphase, with 1 trivalent, 7 bivalents, and 15 univalents; one of the univalents (*u*), lying on the equatorial region, is in the process of division. Such precocious splitting of univalents has so far been recorded only in tetraploid spermatocytes of *Culex* (Moffet, 1936a), although splitting of univalents at first anaphase has been frequently noted. In another cell from this plant some of the metaphase chromosomes clearly showed the second division splits (Fig. 37); in this cell the chromosome complement was 1 trivalent, 6 bivalents, and 17 univalents.

Small chromatin bodies were often seen lying outside the metaphase spindle (Fig. 35, *f*); these, to judge from their size, were chromosome fragments. Instances of bivalents showing arrest of terminalization were not rare; one is figured (Fig. 34, *b*), which suggests that the interstitial chiasma is moving to the ends with difficulty. It has been suggested (Darlington, 1937) that retardation in terminalization may be due to change of homology of chromosomes, while fragmentation of chromosomes has been attributed to the same cause, due to segmental interchange (Philp and Huskins, 1931). The occurrence of these two phenomena in *binervosum*/3 thus gives support to the view that some such change has occurred in the chromosome complement of this plant.

The small chromosomes separate easily at anaphase and pass to the poles, but the longer bivalents and bivalents with interstitial chiasmata separate less

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FIGS. 34-9. *L. binervosum*/3. 34. First metaphase; chiasma moving with difficulty at end of one pair (*b*); CA. 35. An irregular first metaphase, with univalents not on the equatorial region and a fragment (*f*); there are two bivalents (*b*) and a trivalent (*tr*); the rest are univalents, except for the fragment; CA. 36. Complement at first metaphase, showing 1 trivalent, 7 bivalents, and 15 univalents; one univalent in the equatorial region is splitting (*u*); CA. 37. Complement at first metaphase showing 1 trivalent, 6 bivalents, and 17 univalents; second division split is seen in some of the chromosomes; CA. 38. First anaphase, with two bivalents lagging among the univalents; CA. 39. First metaphase in an unreduced nucleus, with 32 chromosomes; CA.



48



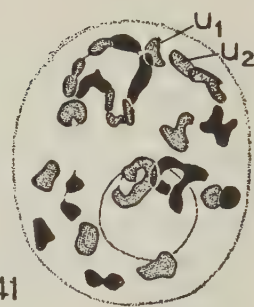
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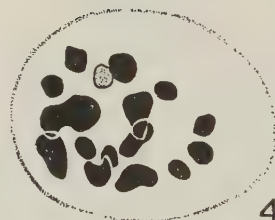
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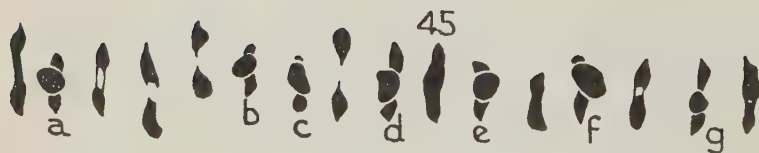
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readily and lag behind the smaller chromosomes along with the univalents (Fig. 38). A similar lagging of bivalents with incompletely terminalized chiasmata has been recorded in other plants (e.g. Moffett, 1936b).

*Binervosum*/39 showed stages between first metaphase and interkinesis in the same anther. In its interkinetic nuclei the wide separation of sister chromosomes, which were held together only in the attachment region, was characteristic. During interkinesis there was a considerable increase in the length of the chromosomes; in certain chromosomes the free ends were invariably observed to lie on the nucleolus (Fig. 33).

The occurrence of giant interkinetic nuclei was another feature associated with *binervosum*/3; these nuclei presumably arose from unreduced gametes with 32 chromosomes, for nuclei with this number of chromosomes were noted (Fig. 39). The occurrence in hybrids and polyploids of such restitution nuclei, with  $2n$  chromosomes, is well known and may be attributed to some irregularity in the first division, such as failure of the chromosomes to orientate on the metaphase plate (cf. Newton and Darlington, 1929), or to fusion of two interphase nuclei.

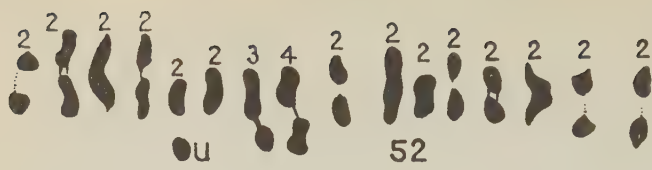
Complete observations on the second division could not be made owing to lack of material. In *binervosum*/3 pollen dyads, triads, pentads, and sextads frequently occurred, as well as tetrads (Figs. 63–8). In such irregular groups the microspores varied in size considerably, the supernumerary nuclei being as large as normal ones or smaller.

(c) *L. vulgare*. At diakinesis in this species there are usually 16 bivalents evenly spread throughout the nucleus. In the larger bivalents interstitial chiasmata were often seen, in the smaller ones the chromosomes lay parallel when the chiasmata were completely terminalized (Figs. 40–2). In the nucleus shown in Fig. 41 there are 13 bivalents, 1 tetravalent ring, and 2 univalents ( $u_1$  and  $u_2$ ) which lie close together; since the other chromosomes have paired these univalents must be homologous, but they have either failed to pair at an earlier stage or have separated precociously; the former alternative seems more probable, and failure to pair due to the presence of too many homologous chromosomes in the nucleus (cf. Darlington, 1937). The tetravalent shown in Fig. 42 with interstitial chiasmata is an exceptional configuration.

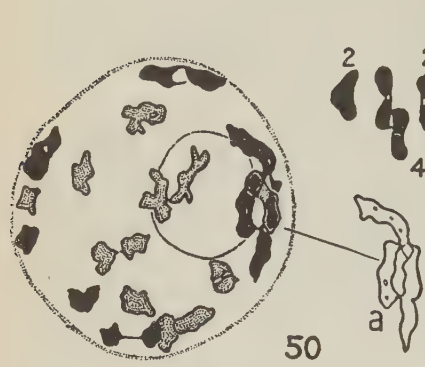
Normally at metaphase there are 16 bivalents at the equator (Fig. 43), and these show different stages of terminalization (Figs. 44, 45), due, presumably,

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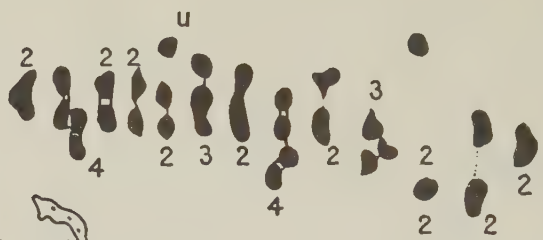
FIGS. 40–9. *L. vulgare*. 40. Diakinesis, with 16 bivalents. 41. Diakinesis, with a ring of 4 chromosomes, 13 bivalents, and 2 univalents ( $u_1$  and  $u_2$ ). 42. Diakinesis, showing tetravalent ring with interstitial chiasmata; nucleus incomplete. 43. First metaphase, with 16 bivalents. 44. First metaphase; chromosome complement, seen laterally, and showing a ring of 4 chromosomes and 14 bivalents; the ring is orientated in a parallel manner; CA. 45. First metaphase complement, showing 16 bivalents, some, *a, b, c, d, e, f, g*, show incomplete terminalization; CA. 46. A trivalent chain from a first metaphase. 47. First metaphase, showing univalent outside equatorial region. 48. First metaphase, showing 2 univalents which have failed to orientate themselves in the equatorial region. 49. First telophase, with lagging univalent.



51

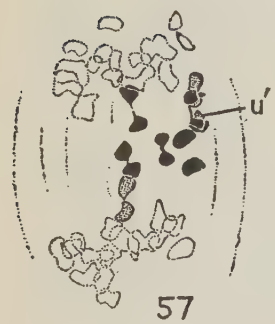


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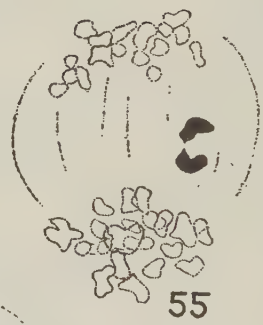
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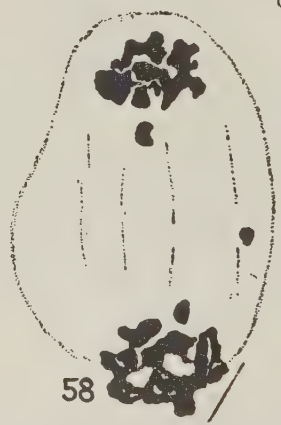
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b

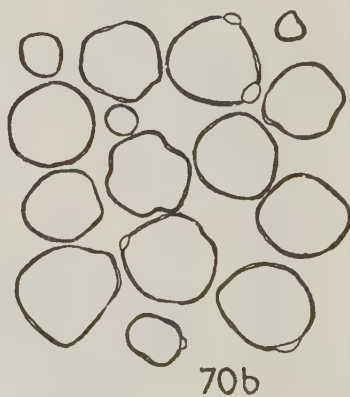
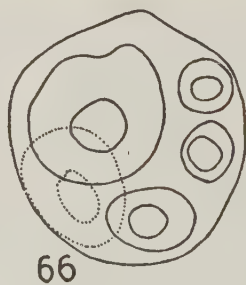
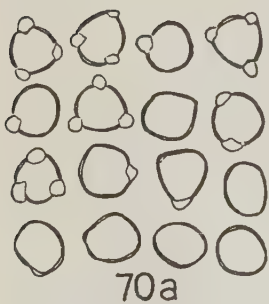
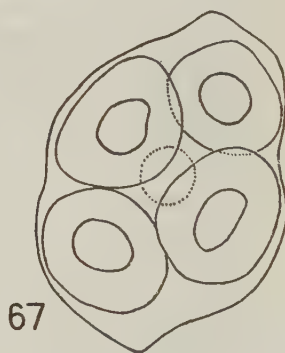
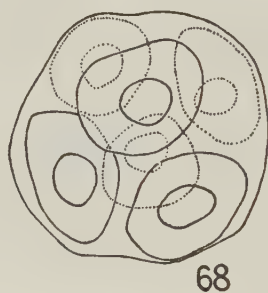
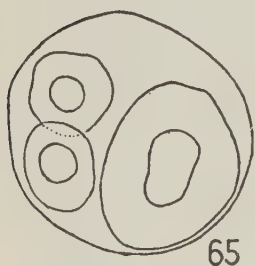
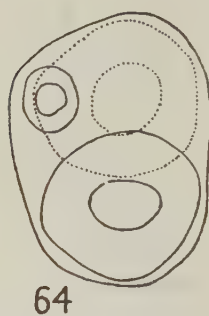
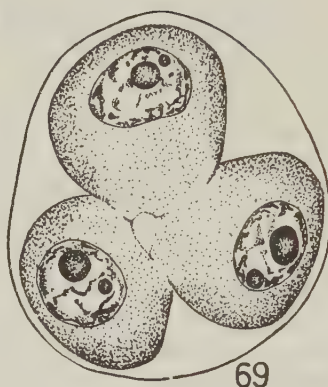
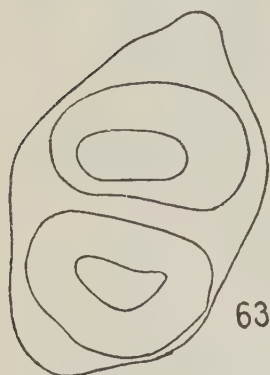
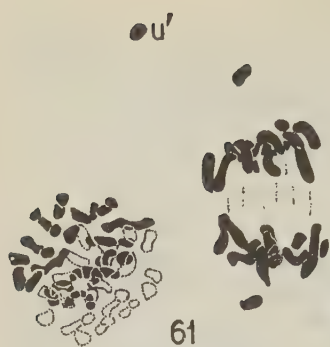
to the process being interrupted by the onset of metaphase (cf. Erlanson, 1931). In Fig. 44 the tetravalent ring and the 14 bivalents are mostly attached by terminal associations, and the tetravalent ring is orientated on the equator in a parallel manner (cf. Darlington, 1937; Parthasarathy, 1939). In many nuclei, of course, the chromosome complement is represented by bivalents only; thus the complement shown in Fig. 45, taken from the same anther as that nucleus which has just been considered, shows 16 bivalents, 7 of which (*a-g*) show incomplete terminalization. Occasionally trivalents were present in this species (Fig. 46), and as a consequence of this uneven association the odd univalent may fail to reach the equator at metaphase. Owing to the density of staining considerable difficulty was found in separating the metaphase chromosomes, but univalents were easily recognized, since they failed to take their place at the equator at metaphase (Figs. 47, 48), and lagged at first anaphase (Fig. 49).

(*d*) *L. rariflora*. While the haploid chromosome number of this species is 18, at diakinesis there may be fewer than 18 groups of chromosomes owing to the formation of trivalents and tetravalents. These multivalent groups were held by terminal associations, and generally took the form of a chain (Fig. 51), although other configurations were occasionally seen; thus in a nucleus figured (Fig. 50) there are 16 bivalents and a tetravalent, in which two of the chromosomes are attached, one at either end, to a ring bivalent. In Fig. 51 two trivalent associations from different nuclei are figured.

Where, in a metaphase, trivalents occurred, the corresponding univalents were to be seen (Fig. 52); in this figure, besides the trivalent and univalent there are 14 bivalents and a tetravalent. In favourable instances centromeres could be detected, orientated along the long axis of the spindle (Fig. 54). Not infrequently the number of univalents in a nucleus appeared to be greater than would be expected from the number of trivalent associations, but it is difficult to ascertain the number of bivalents at this stage with certainty, since the chromosomes of small bivalents may separate precociously and become indistinguishable from the univalents. Evidence of such precocious separation of small chromosomes is seen in Fig. 53, where two chromosomes are passing to the

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FIGS. 50-60. *Limonium rariflora*. 50. Diakinesis; shows one exceptional tetravalent (*a*), with one chromosome attached on either side of a ring bivalent; there are also 16 bivalents. 51. Trivalents from different nuclei at diakinesis. 52. First metaphase complement, showing 1 tetravalent, 1 trivalent, 14 bivalents, and 1 univalent. 53. First metaphase complement, showing 2 tetravalents, 2 trivalents, 10 bivalents, and 2 univalents; one chromosome pair has separated early and is moving toward the poles. 54. First metaphase chromosomes, a trivalent and a bivalent, showing axial orientation of centromeres. 55. First telophase showing 17-18 distribution, with a split, lagging univalent. 56. First anaphase; 16 chromosomes are passing to either pole, and 4 univalents and a half-univalent (*u'*) are lagging; one pole is incomplete and the missing chromosomes (*b*) were drawn from the next section; *a*, chromosomes which have reached pole in advance of the others. 57. First telophase, showing 15-14 distribution, with 7 univalents and a half-univalent (*u'*) lagging; the univalents are all splitting. 58. First telophase, with lagging chromosomes near to their respective poles, and a half-univalent near the equator. 58*a*. First telophase nucleus with 18 chromosomes. 59. First telophase, with a fragment and a small supernumerary body, broken from a fragment, lying at the equator. 60. Interkinesis, with sister chromosomes held together only at centromere.





poles, while others are still at the equator; since these two chromosomes are in the same line, and also equidistant from the equator, they may reasonably be regarded as members of a bivalent which have separated in advance of the others; such chromosomes may reach the poles in advance of the rest of the complement. Larter (1932) records the separation of the smallest chromosome pair in *Ranunculus acris* as soon as the bivalents are orientated on the metaphase plate, but these precociously separated chromosomes pass to the poles along with their fellows.

When bivalents and multivalents disjoin at anaphase, the univalents lag behind on the divisional plane. Univalents usually divide during this division, but may reach the poles without dividing. Lagging of univalents is shown in Fig. 55, where there is a 17-18 distribution of the chromosomes with a divided univalent lagging near the equator. In an exceptional instance (Fig. 57) 7 univalents and a half-univalent were noted; since there are 15 chromosomes at one pole and only 14 at the other, it may reasonably be assumed that a half-univalent lies at the pole with 15 chromosomes. In another nucleus (Fig. 56) there were 4 univalents and a half-univalent at the equator, and 16 chromosomes passing to either pole; the most likely explanation of this figure is that there were 15 paired chromosomes and 6 chromosomes which failed to pair, and that one anaphase group included a univalent, the other a half-univalent. At this time some of the chromosomes may reach the poles earlier than others (Fig. 56, *a*), and it seems probable that those which arrive early are chromosomes which have separated precociously. During anaphase the sister halves of the chromosomes, especially of the large ones, are widely separated except at the centromere region.

The univalents pass towards the poles at late anaphase (cf. Fig. 58); in this figure the small chromosome at the equator probably represents a half-univalent. Density of staining often made it impossible to distinguish individual chromosomes at the poles, although in a few favourable instances 18 chromosomes were counted (cf. Fig. 58*a*).

In *L. rariflora* there is a pronounced resting period, between the first and second divisions, during which the chromosomes lengthen appreciably. At this stage the chromosomes are widely separated except at the attachment regions (Fig. 60).

Few second metaphases were observed, a fact which may indicate that this stage is of short duration. While regular separation occurs in second anaphase, irregularities were frequently encountered. Thus in Fig. 61 two sets of 19

FIGS. 61-70. 61. *L. rariflora*, second anaphase; in the left-hand nucleus 19 chromosomes are separating (the set at the lower focus is in outline); note eliminated part chromosome (*u'*), and in right-hand nucleus early movement of half-univalents. 62. *L. rariflora*, second telophase; left-hand nucleus shows chromatid bridge and a large laggard chromosome. 63-8. Abnormal pollen development in *L. binervosum*/3: 63, dyad; 64, dyad and microcyte; 65, monad and 2 microcytes; 66, dyad and 3 microcytes; 67, tetrad and micronucleus; 68, sextad; Figs. 63-8  $\times c. 1,100$ . 69. *S. bellidifolium*, delimitation of microspores.  $\times c. 2,040$ . 70*a*. *L. bellidifolium*, pollen  $\times 105$ . 70*b*. *L. rariflora*, pollen, including dwarf microspores.  $\times 105$ .

chromosomes are seen on the obliquely lying spindle on the left, while in the spindle which is seen laterally one chromosome (possibly a half-univalent) has reached the pole in advance of the others. In this figure the small chromatin mass ( $u'$ ) in the cytoplasm appears, from its size, to be a half-univalent which was eliminated from the first division. The presence of the 19 chromosomes may be due either to non-disjunction of multiple chromosomes (cf. Kulkarni, 1929) or to splitting of univalents.

An abnormality is figured (Fig. 62) where a chromatid bridge and a large lagging chromosome occupy the equator. The fragment freed by the bridge is not visible, being either obscured by other chromosomes or carried to one pole during the passage of the other chromosomes.

Bridge formation may be explained on the basis of crossing over within an inversion, and the inversion loop observed in *L. rariflora* (Fig. 19, *b*) indicates that bridge formation is a possibility in this species. Crossing over in the inverted region is evidently very infrequent, since bridges are of rare occurrence, while the presence of the bridge in the second meiotic division indicates that the continuous chromatid passes to the same pole in the first division and consequently forms a bridge in the second. Similarly, the presence of fragments but no bridge in the first division, and the presence of a bridge in the second division, suggests that the looped type of configuration (cf. Smith, 1935, in *Trillium*) may occur in *Limonium*, although it has not been noted in the present investigation; a looped type of configuration is due to the presence of two cross-overs, one within the inversion and the other proximal to it, involving one chromatid in both cross-overs. In such a configuration the loop would, of course, pass to one pole in first division, leaving the fragment on the divisional plane, while disjunction would occur at second division.

#### *Meiosis in the Limonium Hybrids.*

(a) *Broad-leaved forms of L. binervosum* (= *L. bellidifolium*  $\times$  *L. binervosum*). The examination of the meiotic divisions of the broad-leaved forms of *L. binervosum* has confirmed the suggestion made by de Fraine and Salisbury (1916) that these plants are hybrids between *L. bellidifolium* and *L. binervosum*.

Little attention could be given to the earlier stages of meiosis owing to poor fixation, a feature which other investigators (e.g. Clausen, 1926) have associated with hybridity; nevertheless, it should be noted that fixation of these early stages was not good in certain species of *Limonium* (cf. p. 193). There was a number of distorted cells, as well as many pollen mother-cells which could not be differentiated in staining, and these appeared as more deeply stained bodies in the anthers; such pollen mother-cells were sometimes observed only in one pollen sac of an anther. Another character associated with these plants was the considerable variation in size of pollen mother-cells at the same stage of division; this feature is not to be attributed to differential contraction brought about by poor fixation. In the smaller pollen mother-cells the chromosomes were more irregular in their behaviour than were those

of the larger cells. Clausen (1926) found a similar size variation in pollen mother-cells in *Viola tricolor*  $\times$  *V. arvensis*, and noted the greater regularity of division of the larger cells, while Meurman (1928) observed very large pollen mother-cells in sterile *Ribes* hybrids, these cells being surrounded on three sides by tapetum and having larger chromosomes than the more normal-sized pollen mother-cells. Both authors attribute such abnormalities to nutritional differences.

The parents of the hybrid *L. binervosum*  $\times$  *L. bellidifolium* have 16 and 9 chromosomes respectively in their haploid nuclei. On the whole, allosyndetic pairing occurs, generally resulting in 9 bivalents and 7 univalents, and it may reasonably be assumed that all 9 of the *bellidifolium* chromosomes pair with 9 of the *binervosum* complement, while 7 *binervosum* chromosomes remain unpaired. The number of bivalents, however, is not constant, and where there are fewer than 9 bivalents there is a corresponding increase in the number of univalents. In some nuclei unexpectedly low numbers of univalents were observed, but in all probability some were obscured by the bivalents.

As evidence that the pairing is allosyndetic, it should be mentioned that the chromosomes of a bivalent are often of unequal size (Fig. 76, *a, b*). In one nucleus (Fig. 76) the chromosome complement at metaphase consisted of 1 tetravalent, 1 trivalent, 8 bivalents, 2 univalents, and 1 fragment, and it is evident that here autosyndesis had occurred among certain chromosomes. The presence of a tetravalent is unusual, and must be regarded as pointing to an abnormal constitution of the chromosomes, for this hybrid may be regarded as a triploid. The tetravalent association is explicable on the assumption that segmental interchange had occurred between two non-homologous chromosomes. Some idea of the range of the chromosome complement in this hybrid will be gathered from the analysis of the metaphase complements of ten pollen mother-cells (Table I).

TABLE I

Metaphase complement in broad-leaved forms of <i>L. binervosum</i> .	Number of pollen mother-cells.	
9 bivalents, 7 univalents (and 1 fragment)* .	6	(cf. Fig. 74)
8 bivalents, 9 univalents (and 1 fragment)† .	3	(cf. Fig. 75)
1 tetravalent, 1 trivalent, 8 bivalents, 2 univalents, (and 1 fragment) . . . . .	1	(cf. Fig. 76)

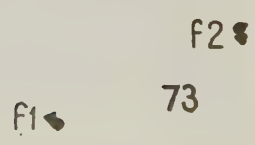
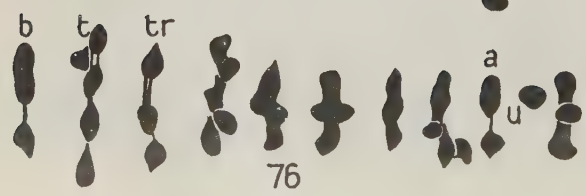
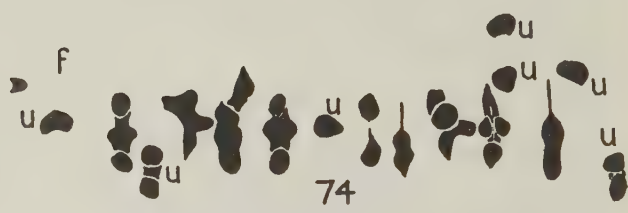
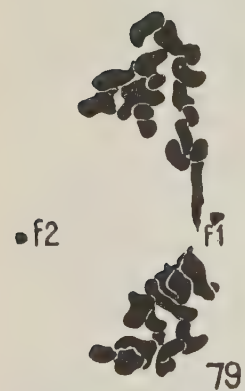
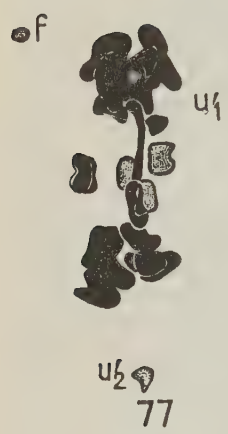
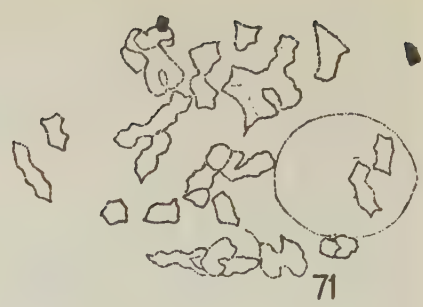
\* In 2 of the 6 pollen mother-cells.

† In 2 of the 3 pollen mother-cells.

At metaphase the univalents do not occupy the equator but lie to either side of it, so that in an oblique view the univalents appear to occupy the periphery and the bivalents the central region (Fig. 72).

Fragmentation of the chromosomes frequently occurs at metaphase; it may even be seen at diakinesis (Fig. 71). These fragments often divide (Fig. 73). If the view of Darlington (1929) be accepted, that fragmentation is usually







connected with abnormalities in segmental homology, then the presence of fragments in *Limonium* affords some justification for the interpretation of the multiple association of chromosomes as due to segmental interchange (cf. p. 193).

At anaphase the disjoined bivalents pass toward the poles, but the univalents at first remain midway between them. The behaviour of the univalents varies; they may split, while still lagging behind the bivalents, and the variable number of univalents in the region of the divisional plane is probably due to some of the univalents reaching the poles divided or undivided, while others lag near the equator (Figs. 77, 78, 82). This may, of course, lead to unequal distribution of the chromosomes to the two poles. Thus in Fig. 82 there are 11 and 13 chromosomes on opposite sides of the equator, and 5 univalents splitting in the divisional plane.

At this time univalents are often eliminated into the cytoplasm. In one instance (Fig. 81) a large chromatin body was noted close to the cell-wall, and this is regarded as a bivalent in which the chromosomes have not disjoined. If this interpretation be correct, then undisjoined bivalents may likewise be eliminated; but such instances of non-disjunction are rare.

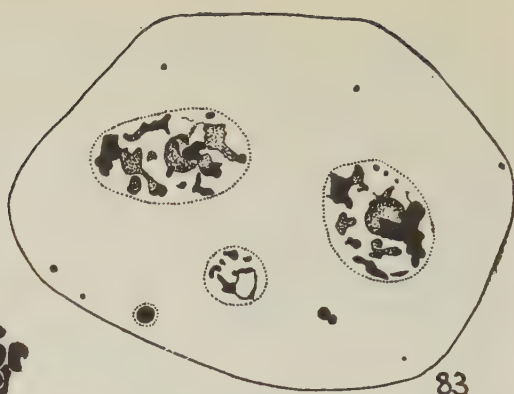
It may happen that the chromosomes of a bivalent separate with difficulty (Fig. 80), and as a consequence its chromosomes lag behind the rest between the two telophase groups; such belated separation of the chromosomes of a bivalent is, however, quite distinct from bridge formation (cf. Figs. 79, 80). In this hybrid telophase is often characterized by the presence of chromatid bridges and fragments. A typical bridge is shown in Fig. 77, together with six undivided univalents, a divided univalent ( $u'_1$ ,  $u'_2$ ) and a fragment ( $f$ ). At a slightly later stage the bridge may break (Fig. 79), and in breaking it may give rise to fragments of different sizes (Fig. 79,  $f_1$ ,  $f_2$ ). Fragments are also produced in some other way, however, since they occur in nuclei with unbroken bridges. In a good many instances the bridge persists, and this leads at times to the formation of a nuclear membrane enclosing both polar groups and the bridge (Fig. 85), and to the subsequent formation of un-reduced gametes.

First telophase is succeeded by a resting period, and the interphase nuclei

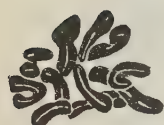
FIGS. 71-80. Broad-leaved forms of *L. binervosum*. 71. Diakinesis, two fragments shown in black; in drawing chromosomes have been more widely spaced than in nucleus. 72. Oblique view of metaphase, in which 11 univalents, shown in black, appear to encircle the bivalents, shown in outline. 73. Chromosomes at first metaphase, and two small fragments ( $f_1$ ,  $f_2$ ) one of which has split. 74. First metaphase complement, with 9 bivalents, 7 univalents, and fragment ( $f$ ). 75. First metaphase complement with 8 bivalents and 9 univalents. 76. First metaphase complement with 1 tetravalent, 1 trivalent, 8 bivalents, 2 univalents, and 1 fragment ( $f$ ); in the bivalents  $a$  and  $b$  the chromosomes are of unequal size. 77. First telophase, with chromatid bridge; 6 univalents and a half-univalent ( $u'_1$ ) are between the telophase groups, while another half-univalent ( $u'_2$ ) and a fragment ( $f$ ) lie outside the spindle zone. 78. First telophase with 7 undivided lagging univalents and an eliminated fragment ( $f$ ). 79. First telophase with two fragments ( $f_1$ ,  $f_2$ ), of unequal size produced by disruption of bridge. 80. First telophase, with a bivalent, in which the chromosomes have separated with difficulty, lagging;  $f$ , fragment.



81



83



82



$f_2$

$f_1$

$\circ f$



86

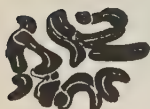


85

$\circ f$



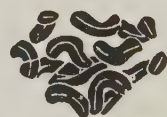
89



88



84



87

differ in size, due, presumably, to the varying number of chromosomes which they contain. The small, supernumerary chromatin bodies which are found in some cells at interkinesis and later (Figs. 83, 92) are probably formed from the breaking up of chromosome fragments; their small size would seem to preclude the assumption that they represent the initial fragments. Eliminated univalents and half-univalents usually form micronuclei; more rarely entire chromosomes may be left between the telophase groups to form larger micronuclei (Fig. 83).

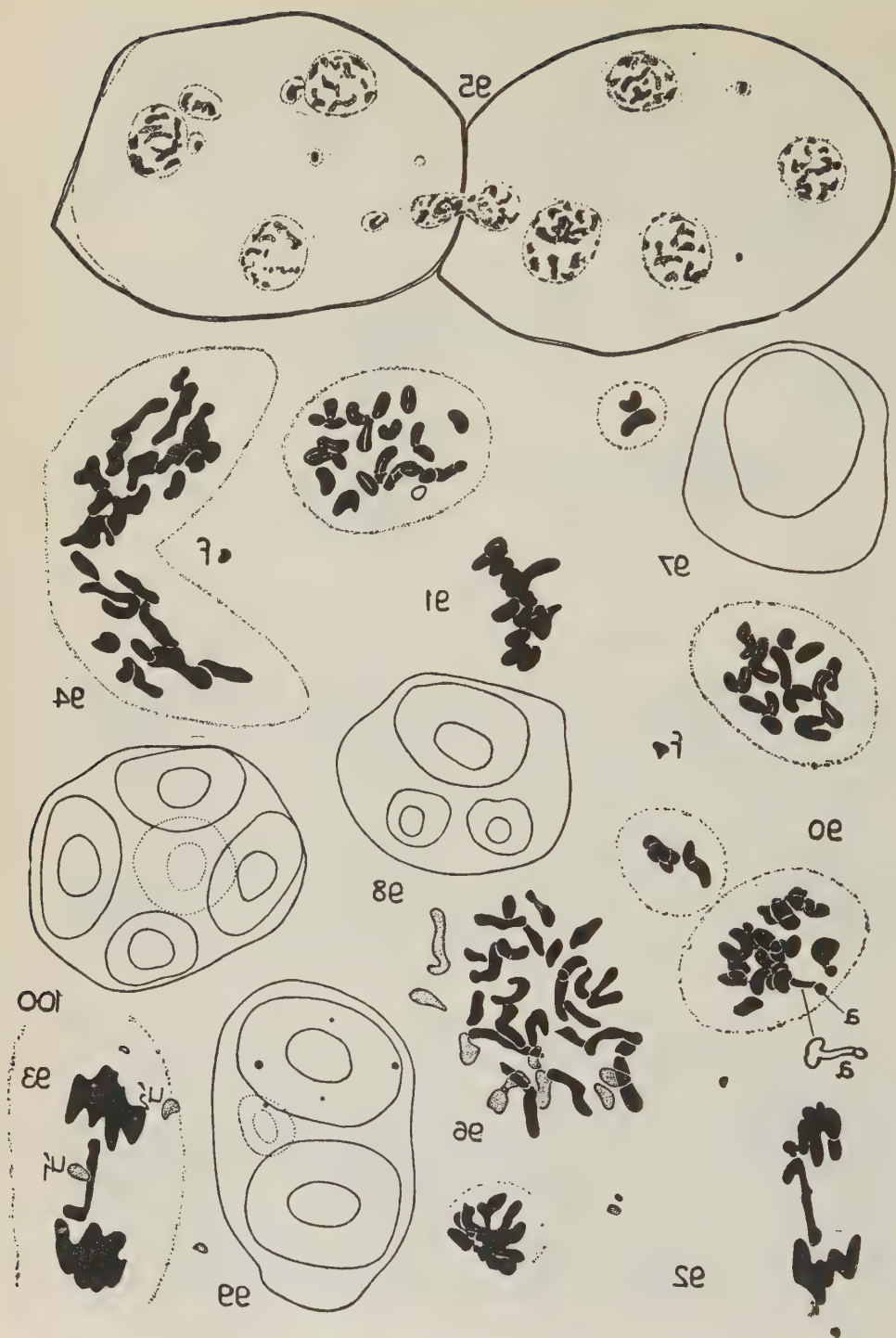
It has already been noted that the distribution of the chromosomes in the first division was irregular, owing to the behaviour of the univalents, and as might be expected, a similar irregularity was found in the second division anaphase. Table II gives some indication of the extent of this unequal distribution.

TABLE II

Chromosome number on second metaphase plates in broad-leaved forms of <i>L. binervosum</i> .		Eliminated chromosomes.	Figure.
12	13	..	86
12	12	1	87
11	13	1	88
13	15	3	90
16 and		2	91
1 fragment		(fragment shown in outline)	

There can be no question that the univalents divide before second metaphase in some instances, since the number of chromosomes at second metaphase may be unusually high (Figs. 90, 91). In *Pygaera* hybrids Federley (1913) observed division of univalents at first metaphase and consequent increase in the number of chromosomes, sometimes to double the expected number, while Clausen (1926) found division of univalents at anaphase in *Viola* hybrids. The attenuated chromosome shown at *a* in the metaphase plate in Fig. 90 is probably to be interpreted as a chromosome which contributed to a bridge in the first division, and which would have separated during second anaphase. Fragments are generally eliminated, but the small chromatin body

FIGS. 81-9. Broad-leaved forms of *L. binervosum*. 81. First telophase; one pair of split univalents is separating; two half-univalents and a whole chromosome have been eliminated and lie against the cell-wall. 82. First telophase, showing 11-13 distribution, with the 5 univalents splitting at the equator: an excluded fragment is not shown. 83. Interkinesis, with micronuclei formed from eliminated part chromosomes; the large micronucleus has been formed from a whole eliminated chromosome; minute supernumerary chromatin bodies are present, formed from the breaking up of fragments. 84. A giant interkinetic nucleus formed by the fusion of two daughter nuclei. 85. Formation of a giant nucleus due to the persistence of the bridge. 86. Second metaphase plates showing 12 and 13 chromosomes respectively; *f*, fragment. 87. Second metaphase plates showing 12 chromosomes each, while another chromosome has been eliminated; *f*, fragment. 88. Second metaphase plates showing 11 and 13 chromosomes respectively, while another chromosome has been eliminated. 89. Second metaphase showing 12 and 14 chromosomes respectively, and two fragments (*f*<sub>1</sub>, *f*<sub>2</sub>).





shown in outline in Fig. 91, which from its size can only be interpreted as a fragment, indicates that at times fragments reach the poles, probably carried there by separating chromosomes.

An important feature which is to be observed in chromosomes at second metaphase and subsequently is that they are double (Figs. 86-91).

Second division anaphases are more regular than those of the first division, and the spindles lie either parallel to or at right angles to the plane of the first division. Micronuclei formed round chromosomes eliminated during first division may be included in second division groups at this time if they lie near the poles of the spindles. Not infrequently second division spindles lie obliquely to one another, so that two of the poles may be in contact (Fig. 94); as a result the two spindles may fuse and in this way unreduced gametes are produced.

Chromatid bridges were frequently seen at second division telophase (Figs. 92, 93). In Fig. 93, in addition to the bridge, two small chromosomes are seen ( $u'_1$ ,  $u'_2$ ), which have probably arisen by division of a univalent during second anaphase.

In Fig. 95 an unusual example of nuclear migration is shown, a late telophase nucleus passing from one pollen mother-cell into the adjacent one. Similar nuclear migrations were noted by Woodworth (1929) in most species of the Betulaceae which he investigated. Such apparent nuclear migrations in the earlier stages of division are generally held to be artifacts produced by fixation, but such a view is regarded as untenable as far as the material under present consideration is concerned. There is no reason to suppose that, in *Limonium* species, or in the hybrids, fixation is poor at this stage. Nevertheless, irregularities do occur in hybrids, with the resulting formation of monads, dyads, and pentads as well as tetrads (Figs. 97-100). The formation of dyads is frequent; they appear to arise by the omission of the second division, and the gametes formed have 32 chromosomes (Fig. 96). Although, as might be expected, the pollen grains vary in size (Fig. 115), there is no visible indication of sterility among them.

(b) *L. Neumani* = (*L. vulgare*  $\times$  *L. rariflora*). *L. Neumani* has for a long time been regarded, with good reason, as a hybrid between *L. vulgare* and

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FIGS. 90-100. Broad-leaved forms of *L. binervosum*. 90. Second metaphase plates, showing 13 and 15 chromosomes respectively; 3 eliminated chromosomes form a large micronucleus; 1 chromosome (*a*) is much elongated, while a fragment (*f*) lies between the plates. 91. Second metaphase with 16 chromosomes and a fragment (in outline) in one plate; 2 eliminated chromosomes form a micronucleus. 92. Second telophase; note that the two chromosomes forming bridge are dissimilar; broken fragments form several supernumerary chromatin bodies. 93. Second telophase, with bridge, a lagging half-univalent ( $u'_1$ ) and an eliminated half-univalent ( $u'_2$ ). 94. Second anaphase; an abnormality; shows fusion of two obliquely placed spindles; *f*, fragment. 95. Second telophase, showing migration of microspore nucleus into adjacent cell; there are several micronuclei formed from eliminated whole or part chromosomes ( $\times$  c. 1,660). 96. Metaphase of first division in a pollen grain, with 32 chromosomes. 97-100. Abnormal pollen development ( $\times$  c. 1,100): 97, monad; 98, monad and 2 microcytes; 99, dyad and microcyte; 100, pentad.

*L. rariflora*. In *L. vulgare* the haploid number of chromosomes is 16, in *L. rariflora* 18.

Pairing in the hybrid is allosyndetic, the 16 *vulgare* chromosomes pairing with 16 of the *rariflora* chromosomes; this results in 16 bivalents and 2 univalents, but as complete allosyndetic pairing does not always occur, the number of bivalents, and consequently of univalents, is somewhat variable. The univalents fail to orientate themselves on the equatorial plate at first division metaphase, and lie on either side of the equator.

An analysis of first metaphase groups to show the frequencies of bivalents and univalents is given in Table III.

TABLE III

Metaphase complement in <i>L. Neumani</i> .	Number of pollen mother-cells.	Figures.
16 bivalents, 2 univalents . . . .	8	101, 103
15 bivalents, 4 univalents . . . .	3	104
14 bivalents, 6 univalents . . . .	1	102, 105
12 bivalents, 10 univalents . . . .	1	106
	<hr/> 13	

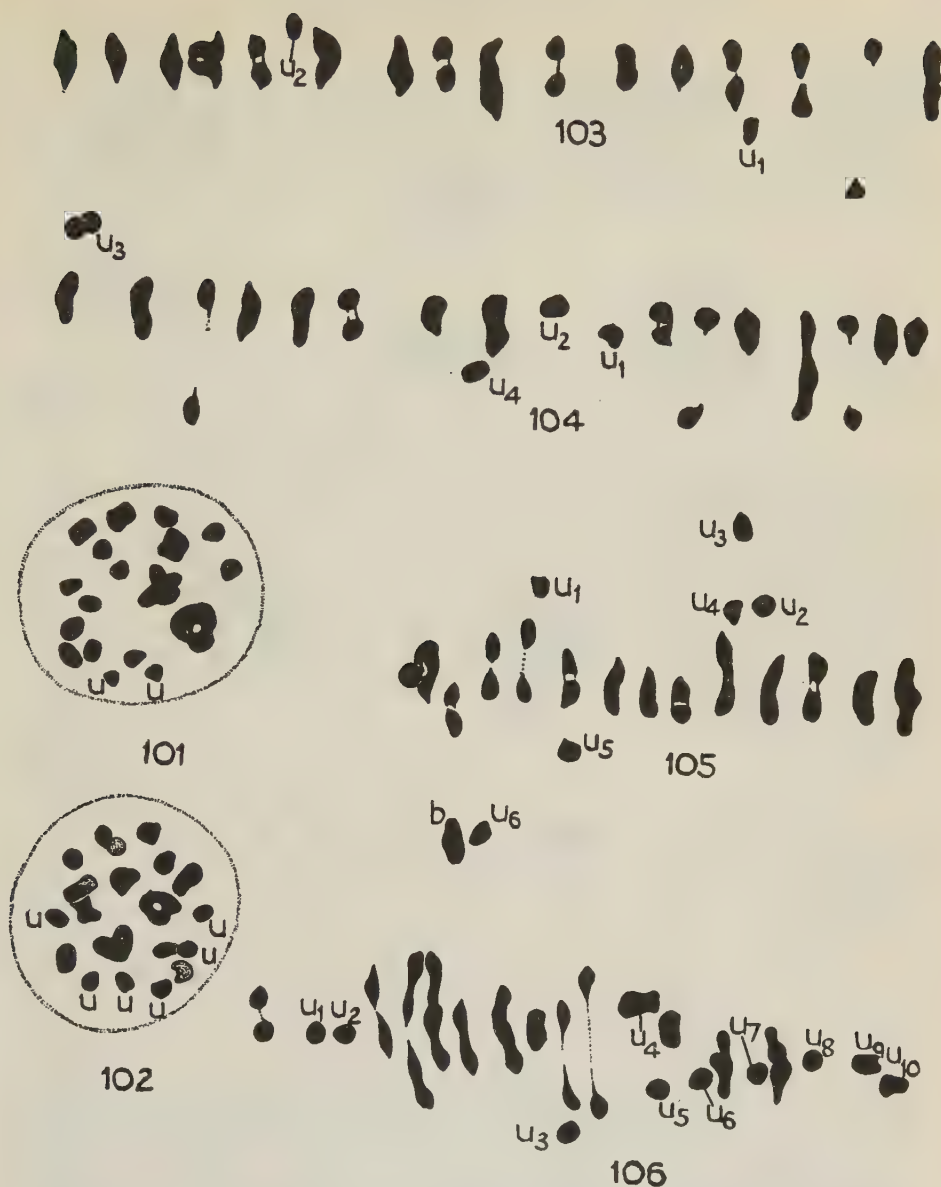
It will be noted that in Fig. 105 one of the bivalents, like the 6 univalents, has failed to take up its position in the equatorial region, although 13 bivalents are thus orientated. This behaviour recalls that noted by Church (1929), in *Paspalum muhlenbergii* and in several species of *Panicum*, where lagging and extrusion of bivalents has been correlated with known or suspected hybrids.

At anaphase the bivalents separate normally, leaving the univalents in the equatorial region. The univalents split, but their passage to the poles may not be regular, and uneven distribution is often seen. Fig. 107 shows 16 chromosomes in a pole with two lagging univalents, while in Fig. 108 15 chromosomes are shown in a polar view of first division telophase.

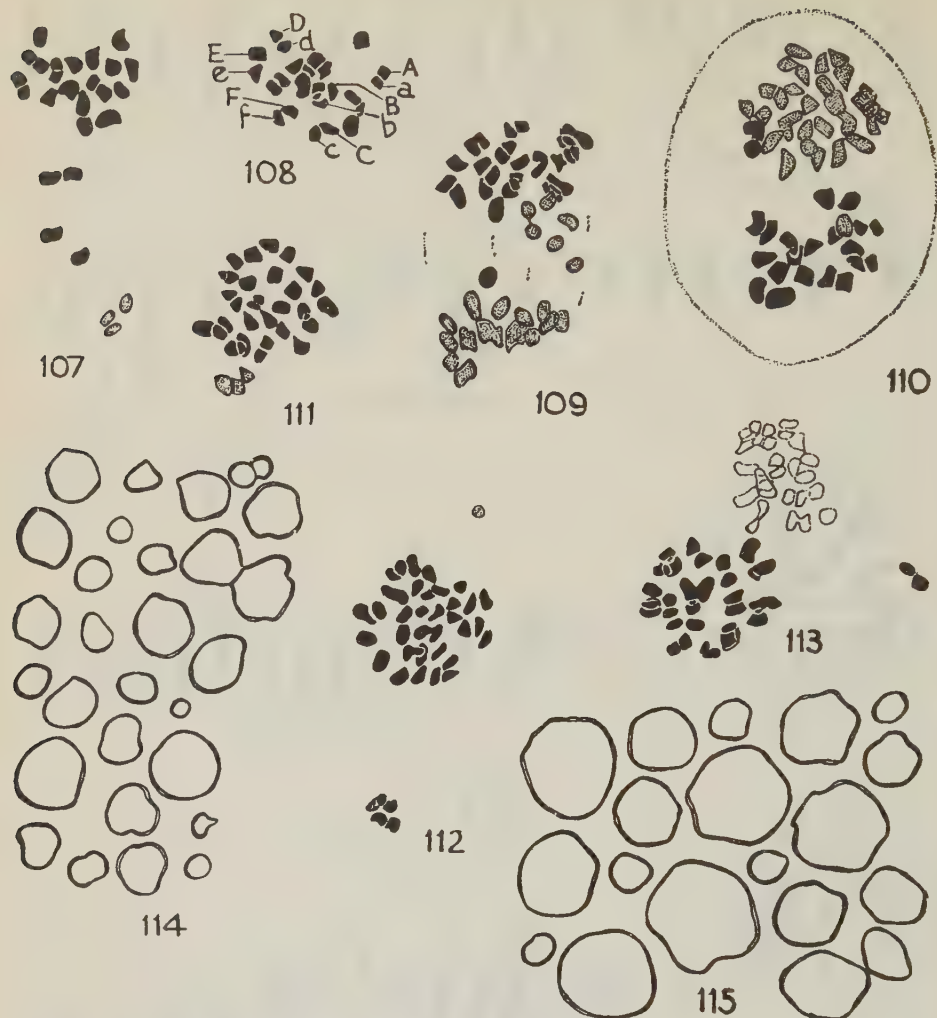
At telophase the doubleness of the chromosomes is apparent, and sister chromosomes, while they lie in close proximity, are often separated from one another completely (Fig. 108, *Aa-Ff*).

In one instance a pollen mother-cell was noted with 17 chromosomes at one pole and 14 at the other (Fig. 109); this irregularity is interpreted as due to non-disjunction, although no bivalent could be discovered in the group of 17 chromosomes; this cell had also 3 split univalents and a half-univalent lagging between the telophase groups; the other half-univalent was included in the pole with 17 chromosomes, hence the increased number. Similar reports of non-disjunction are frequent in hybrids (cf. Church, 1929).

In some telophases unusually high chromosome numbers were found; thus one pollen mother-cell showed 23 chromosomes at one pole and 16 at the other (Fig. 110), another 25 chromosomes and 3 half-univalents (Fig. 111). Chromosomes are also frequently eliminated during division; thus in the cell



FIGS. 101-6. *L. Neumani*. 101. First metaphase, showing 18 chromosomes, presumably 16 bivalents and 2 univalents. 102. First metaphase, showing 20 chromosomes, presumably 14 bivalents and 6 univalents. 103. First metaphase complement showing 16 bivalents and 2 univalents ( $u$ ). 104. First metaphase complement showing 15 bivalents and 4 univalents ( $u$ ). 105. First metaphase complement with 14 bivalents and 6 univalents ( $u$ ); one bivalent ( $b$ ) has failed to orientate itself on the equator. 106. First metaphase complement with 12 bivalents and 10 univalents ( $u$ ).



FIGS. 107-115. 107-114: *L. Neumani*. 107. First telophase (incomplete); one pole shows 16 chromosomes, and the 2 univalents have split. 108. First telophase, with 15 chromosomes; the chromosomes are split and are labelled *A, a, B, b, . . .*; in certain pairs sister chromosomes are widely separated. 109. First telophase with 17 and 14 chromosomes, respectively; there are 3 split univalents and a half-univalent lagging. 110. First telophase with 23 and 16 chromosomes, respectively. 111. First telophase with 25 chromosomes; 3 half-univalents have not been included in the polar group. 112. First telophase with 31 chromosomes; 2 split univalents and a half-univalent eliminated. 113. First telophase with 18 and 15 chromosomes, respectively, and an eliminated split univalent. (In the drawing the chromosomes are more widely separated than in the nucleus.) 114. Pollen, showing variation in size and dwarf grains ( $\times 105$ ). 115. Broad-leaved *L. binervosum*, pollen showing variation in size and dwarf grains ( $\times 105$ ).



shown in Fig. 112 two split univalents and a half-univalent have been eliminated, while the unusually high number of 31 chromosomes is seen at one pole; in Fig. 113 the distribution is 18-15, with one eliminated chromosome, while in another the distribution was 18-20. Unusually high chromosome numbers have no doubt arisen by splitting of one or more chromosomes; they have been recorded on a number of occasions (e.g. *Pygaera* hybrids (Federley, 1913), *Viola* hybrids (Clausen, 1926), hybrid *Saccharum* (Bremer, 1923)).

### Pollen.

Pollen formation in the diploid *L. bellidifolium* is very regular (Fig. 69). In *L. rariflora* dwarf pollen is occasionally formed, while in the hybrids *L. Neumani* and the broad-leaved forms of *L. binervosum* the formation of extra nuclei from eliminated part or whole chromosomes (*polycary*) is frequent. The production of more than four pollen grains by a pollen mother-cell (*polyspory*) has, however, not been observed in *L. Neumani*. In both hybrids the unusual condition exists of apparently perfect pollen associated with polycary (Figs. 114, 115), but it should be emphasized that the viability of the pollen has not been tested. Neuman (quoted from Salmon, 1904) mentioned the finding of apparently perfect pollen in *L. Neumani*, which he had received from England. It would not be wise, however, to assume that the pollen is perfect without tests for viability, for Thompson (1926) has shown that in wheat hybrids the viability of apparently good pollen is low.

## DISCUSSION

(a) *Pairing of polyploids.* In the tetraploid *L. rariflora* pairing can occur between any two of four homologous chromosomes, and any one of the four may pair with different homologues in different parts of its length. In later prophase the homologues may, therefore, separate in pairs, or, as a result of multiple association, three or four may be held together by chiasmata. The formation of quadrivalent rings and subterminal chiasmata noted in the tetraploid *L. vulgare* is explicable only on the assumption of lateral association. The formation or occurrence of similar rings has been noted by several authors (cf. Gairdner and Darlington, 1931).

Pairing in a tetraploid nucleus should, of course, theoretically lead to the production of the haploid number of tetravalents, but in *Limonium* this condition is generally only partially attained. Failure to form tetravalents may be due to mechanical interference with pairing by reason of the presence of too many homologous chromosomes (Gairdner and Darlington, 1931; Moffett, 1936a), and thus explain the presence of univalents, bivalents, and trivalents as well as tetravalents in the tetraploid species of *Limonium*. The low frequency of tetravalents in *L. rariflora* and *L. vulgare* may also be accounted for by the assumption that the number of pairing blocks (Darlington and Mather, 1932) in the homologous chromosomes is small, so that two homologous

chromosomes which have started to pair are likely to continue to do so over a relatively long distance; since the number of chiasmata will be proportional to the length of the paired chromosomes, chiasmata will rarely form in regions paired for a short distance; hence the chances that bivalents will form rather than tetravalents. The time factor must also be considered; pairing in *Limonium* starts at a point and passes, generally regularly, along the chromosomes; thus two chromosomes which have started to pair are more likely to continue this process, since the time for pairing is short, and pairs may remain unpaired at the ends (Fig. 16). This factor may tend to reduce multiple associations owing to the formation of insufficient chiasmata to retain the connexion in later stages. Similar instances of failure to form multiple associations have been noted by several investigators (cf. Gairdner and Darlington, 1931), while the best example is that described by Moffett (1936b) in tetraploid spermatocytes of *Culex pipiens*, where, apart from an occasional trivalent and corresponding univalent, only bivalents are produced.

(b) *Pairing in hybrids, and segmental interchange.* Attention has been drawn to the morphological dissimilarity between members of certain chromosome pairs in the hybrid broad-leaved *L. binervosum* (pp. 200–7); it may, therefore, be inferred that chromosomes of the parent species which are morphologically different are, nevertheless, homologous in certain regions, an inference which is supported by the high frequency of pairing. Avery (1930) has reached a similar conclusion in her studies of certain *Crepis* hybrids.

In the hybrids under present consideration pairing is generally allosyndetic, but in one cell of the hybrid *L. bellidifolium*  $\times$  *L. binervosum* both allo- and autosyndesis were observed. Hence, in the absence of identical, or at least homologous mates, certain chromosomes may pair among themselves. It is also to be noted, however, that the characteristic failure of pairing in hybrids was also observed in the *Limonium* hybrids, and this may be attributed to genetic dissimilarity of the parent chromosomes.

The presence of multiple associations of four chromosomes in *L. bellidifolium*  $\times$  *L. binervosum* may be taken as evidence of segmental interchange between two non-homologous chromosome pairs; in fact, this configuration is explicable on this assumption alone; the rarity of such multiple associations suggests that the interchanged segments are very small, since the number of chiasmata will be proportional to the length of the paired chromosomes and hence chiasmata will be rarely formed between the short interchanged segments; as a consequence the chromosomes would tend to remain in pairs instead of forming a multiple chromosome. But segmental interchange will have led to the formation of chromosome pairs with different segments and consequently to the production of different types of zygote. Hence segmental interchange might be expected to lead to the production of a range of plants differing in the constitution of the chromosome complexes.

Segmental interchange affecting a large part of the chromosomes is associated with sterility (e.g. *Datura* (Blakeslee, 1929), *Drosophila* (Muller, 1930)),

but it is suggested that in *Limonium*, where the segments are small, the fertility of the zygotes is not impaired.

(c) *The chromatid bridge and the possible evolution of new chromosomes.* While chromatid bridges were frequently observed in first and often in second division in the broad-leaved *L. binervosum*, they were not seen in either parent. Such bridges separated with difficulty, and while they sometimes broke before interkinesis, they sometimes persisted until later stages. Fragments of different sizes are produced when the bridge breaks, but since fragments may be seen near bridges, it follows that they may also be produced before breakage occurs.

Chromosomes which contribute to bridge formation are dissimilar, one showing a subterminal and the other a submedian centromere (Figs. 92, 93). Since, however, the chromosomes are associated, it must be concluded that they are homologous in certain regions, in spite of their morphological dissimilarity. Interchange of segments, followed by crossing over in the homologous regions and with the centromeres in different positions, would explain bridge formation and the appearance of a long, continuous chromatid and fragment (Müntzing, 1934; Lamm, 1936). Evidence that segmental interchange may have taken place is found in the presence of fragments at metaphase and even at diakinesis, for fragmentation of chromosomes is often associated with this phenomenon (Darlington, 1929; Philp and Huskins, 1931). Moreover, the occurrence of segmental interchange has been adduced from the presence of multiple chromosomes.

It is unnecessary to discuss here the process leading to the formation of bridges at the first and second division, since it has already been dealt with by several authors, e.g. Müntzing (1934) in *Crepis*, Richardson (1936) in *Lilium*.

As the chromatid bridge may break at any point, and thus give rise to chromosomes with additional segments, or to chromosomes lacking certain segments, new chromosome types may arise in the broad-leaved *L. binervosum*. A chromosome of the latter type might prove lethal to the gamete which contained it, but not of necessity, provided the missing segment were small. New chromosome types would also be increased by crossing-over in different regions of the homologous segments.

Thus interspecific crosses may lead not only to numerical differences in chromosome complements as in *Viola* and *Saccharum*, but also to structural differences and consequently to the appearance of new chromosomes (e.g. *Crepis* (Navashin, 1934)). It seems probable that such structural alterations have occurred in the broad-leaved forms of *L. binervosum* as a result of hybridization.

(d) *Polyploidy in Limonium.* That both auto- and aliopolyploidy have played an important part in the evolution of new species has been demonstrated in a number of genera of plants. From the present investigation and from the work of Sugiura (1936) the basic chromosome numbers in *Limonium*



appear to fall into two classes, one with 8 as the haploid number, the other with 9. Tetraploids, e.g. *L. binervosum*, *L. vulgare*, *L. rariflora*, occur in both classes, and probably owe their origin to duplication of the whole chromosome set. Slight modifications in chromosome segments may also have occurred, without bringing about any visible change in the chromosome morphology (cf. *L. rariflora*). Such changes are not unknown; thus Meurman (1929) has demonstrated segmental interchange in certain chromosomes in the autotetraploid *Aucuba japonica*.

As in other polyploids, *L. vulgare* and *L. rariflora* exhibit irregularities during pollen formation, but these irregularities, such as lagging of univalents, and rarely, elimination of part chromosomes, do not seem to produce much interference with the formation of pollen, although varying numbers of chromosomes in the gametes would be expected; this feature has not yet been fully investigated, although evidence has already been given that the chromosome numbers do vary.

(e) *Limonium hybrids*. This investigation has produced cytological evidence that the broad-leaved *L. binervosum* and *L. Neumani* are hybrids, and it is well established that hybrids are characterized by meiotic irregularities; the question thus arises whether these putative hybrids behave in any way like known hybrids. Failure of pairing among pairable mates, lagging, and elimination of chromosomes are frequent, and coupled with such irregularities may be noted the formation of abnormal pollen—monads, dyads, pentads, and dwarf grains; such features may be regarded as characteristic of hybrids: in fact Jensen (1936) claims that such irregularities may be regarded as certain indications of hybrid origin, while similar views have been expressed by other investigators (cf. Church, 1929; Hicks and Stebbins, 1934; Woodworth, 1929).

Further, it seems logical to argue that strains of *L. binervosum* (cf. *binervosum*/3) which exhibit a considerable amount of irregularity, such as high frequency of univalents and irregularities in pollen formation, are hybrids derived from later generations of *L. bellidifolium*  $\times$  *L. binervosum*.

## SUMMARY

1. The chromosome numbers of the several species and hybrids of *Limonium* investigated are:

	<i>2n</i>	<i>n</i>
<i>L. bellidifolium</i> Dum. . . . .	18	9
<i>L. binervosum</i> Salmon. . . . .	32	16
<i>L. vulgare</i> Mill. . . . .	32	16
<i>L. rariflora</i> O. Kuntze . . . . .	36	18
<i>L. bellidifolium</i> $\times$ <i>L. binervosum</i> (broad-leaved forms of <i>L. binervosum</i> ) . . . . .	—	$9_2 + 7_1^*$
<i>L. vulgare</i> $\times$ <i>L. rariflora</i> ( <i>L. Neumani</i> ) . . . . .	—	$16_2 + 2_1^*$

\* Usually; irregularities occur.



2. *L. binervosum*, *L. vulgare*, and *L. rariflora* are tetraploids.
3. The several species exhibit differences in size and in the morphology of their chromosomes.
4. In meiosis pairing begins at a point and generally passes regularly along the chromosomes. Where four homologous chromosomes lie together, the two outer ones may be prevented from pairing by the pairing of the two middle ones over a long distance. Pairing may be incomplete in segments remote from where it begins.
5. In *L. bellidifolium* meiosis is regular. At interkinesis nucleolar material appears as minute globules which later coalesce to produce a large nucleolus.
6. In *L. binervosum* there are strains which behave irregularly at meiosis; such strains show association of chromosomes in threes and a high frequency of univalents. Unreduced cells with 32 chromosomes occur. Strains showing such irregularities often show the formation of pollen dyads, and also polyspory.
7. In *L. vulgare* trivalents and ring tetravalents occur, although bivalents are much more frequent, and unpaired chromosomes occur at diakinesis.
8. In *L. rariflora* multiple chromosomes are generally in trivalent and tetravalent chains, and the presence of trivalents leads to the occurrence of univalents. Smaller chromosomes often separate precociously and arrive at the poles in advance of the rest, while univalents may lag in the equatorial region before splitting and passing to the poles.
9. In *L. bellidifolium*  $\times$  *L. binervosum* pairing is, on the whole, allosyndetic, and 9 bivalents and 7 univalents are usually formed, although there is considerable variation in this respect. Fragments are frequent at metaphase, and first division telophases are frequently characterized by the presence of chromatid bridges. Persistence of the bridge leads to the formation of unreduced gametes, which may also arise by fusion of spindles at second anaphase. In second division sister division plates may show unequal distribution of chromosomes. Micronuclei are formed from parts of, or more rarely whole, chromosomes, which have been eliminated; such micronuclei may form supernumerary microspores.
10. In *L. vulgare*  $\times$  *L. rariflora* (= *L. Neumani*) pairing is typically allosyndetic, forming 16 bivalents and 2 univalents, but the number of bivalents and univalents is variable. Univalents split at first division, and this leads to unusually high numbers of chromosomes in first division telophases. Micronuclei are frequently formed around eliminated part chromosomes. Dwarf microspores may be formed.
11. In both hybrids there is considerable variation in the size of the pollen, but no visible evidence of sterility.

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# Biological Technique for the Evaluation of Fungicides

## II. The Evaluation of Seed Disinfectants for the Control of Seed-borne Diseases of Flax

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### INTRODUCTION

DURING the course of an investigation of the prevention of stem-break and browning (*Polyspora Lini* Laff.) and seedling blight (*Colletotrichum Lini* (Westerd. Toch.)) of flax by seed disinfection, an attempt was made to develop standard biological technique for the assessment of the fungicides employed. The problem was approached along lines similar to those followed by Muskett (1938) in the evaluation of seed disinfectants for the control of Helminthosporium disease of oats, and the results obtained from experiments made during the period 1939-41 are here presented.

### CHOICE OF SEED SAMPLES

In the first place it was necessary to procure samples of flax seed heavily contaminated with one or other of the two fungi (*P. Lini* or *C. Lini*). The Ulster method as developed by Muskett and Malone (1941) for the examination of flax seed for the presence of seed-borne parasites was used for the selection of such samples. In so far as it was possible the samples were selected as containing a high percentage of seeds infected with either *P. Lini* or *C. Lini* and not with both parasites, and they were used only during the year immediately following that in which the seed had been harvested.

## SEED TREATMENT

In carrying out the dry treatment of seed the required quantity of the fungicide was shaken with the seed for 5 minutes in a stoppered Erlenmeyer flask. In the case of the short-wet treatment the requisite amount of liquid was added to the seed in an Erlenmeyer flask and thoroughly mixed by shaking for 5 minutes.

In order to ensure that as much of the powder as possible should adhere to the seed coat a method was evolved for the adfixing of the powder by the use of a liquid such as separated milk or water. When applying the 'fixation' method the seed was either mixed first with the appropriate amount of powder which was then 'fixed' by adding the requisite quantity of liquid, or the order of treatment was reversed. The fixation method can be described as a combination of both dry and short-wet methods.

## THE LABORATORY METHOD

For dealing with the large number of substances which it was necessary to test as seed disinfectants, a method was devised which permitted of rapid discrimination between likely and unlikely fungicides. The Ulster method, with slight modification, was found to be suitable for the purpose. This method shows that when flax seeds infected with *P. Lini* or *C. Lini* are plated out on 2 per cent. malt agar, the fungus grows rapidly into the medium and the colonies so formed can readily be identified by eye inspection. From this it was argued that if a sample of seed heavily infected with either of these two fungi is disinfected and then examined by the Ulster method, the growth of the fungi would be suppressed by an effective fungicide. Preliminary experiments confirmed this view and showed that the use of seed disinfectants did not interfere appreciably with the operation of the technique. During the carrying out of the earlier experiments it was found that when certain disinfectants were used, the colonies of *P. Lini* sometimes consisted of abundant, densely packed mycelium with relatively sparse spore formation, in place of the typical loose freely sporulating growth spreading out from the seed into the medium. Since these abnormal colonies showed somewhat slower development, the time of incubation of the plates was increased from 5 to 7 days. This modification of the Ulster method, used for the rapid assessment of the values of seed treatments, was introduced into routine practice in 1939.

## THE FIELD METHOD

The field technique employed consisted of sowing disinfected seed under field conditions and making frequent observations on the incidence and spread of disease during the growing season.

The seed-bed was prepared as in normal agricultural practice and a dressing of muriate of potash applied at the rate of 1 to 1.5 cwt. per acre. If the land was inclined to be poor an application of 0.5 cwt. of sulphate of ammonia per acre

was made. Three sizes of experimental plot— $2 \times 2$  yds. ( $1.83 \times 1.83$  m.),  $3 \times 3$  yds. ( $2.75 \times 2.75$  m.), and  $4 \times 12$  yds. ( $3.66 \times 11.0$  m.)—were used and paths 2 yds. ( $1.83$  m.) in width were left between the plots so as to prevent the spread of disease from plot to plot by the intermingling of the plants.

The seed was sown in drills at 6 in. (15 cm.) intervals, and these were prepared by the use of a large rake bearing seven hollow 'V'-shaped teeth. Sowing was carried out at the rate of 80 lb. per acre, the quantity of seed required for each plot being determined by weight. In the case of the smaller plots the seed was sown from paper envelopes while for the plots measuring  $4 \times 12$  yds. a small hand-operated drill was used. The drills were closed with a rake, the land having been well rolled before the marking out of the plots.

The plots were arranged in blocks, each block consisting of a complete experimental series and the position of each plot within the block was selected at random. The number of blocks varied according to whether the trial was made in duplicate, quadruplicate, or quintuplicate.

The time of sowing selected was that normal to the season.

In some years it was found necessary to carry out careful hand weeding of the plots during the growing season.

*Evaluation of seed treatment.* The symptoms of disease caused by *P. Lini* occur in three well-marked phases, viz. the seedling phase, stem-break, and browning (Lafferty, 1921). Observations for the incidence of the seedling phase are best made about one month after sowing. In estimating the effect of seed treatment an assessment figure of 10 was given to the most heavily infected plots, including the controls, and the others were assessed at between 10 and 0 according to the amount of infection present. From the values obtained for the replicates receiving the same treatment the mean value for each treatment was calculated. Observations for stem-break are usually best made about two months after sowing, and the same method of evaluation was employed as for the seedling phase. As soon as browning was observed (usually towards the end of July), the extent of the outbreak in each plot was assessed in the same manner. In order to study the spread of browning as the season advanced, observations were made at weekly intervals from the time of its first appearance until the crop was harvested or until such time as the disease had spread so much as to make further observations unprofitable.

The attack by *C. Lini* is best observed in the seedling phase (Pethybridge and Lafferty, 1918), and the evaluation of seed treatments for preventing this disease was carried out in the same manner as for *P. Lini* and at about one month after sowing.

#### EXPERIMENTAL DETAILS

##### *Stem-break and Browning (P. Lini).*

1939

*Seed samples.* Two seed samples of the variety Stormont Cirrus were used;

the percentage number of seeds infected with *P. Lini* in each were 17.6 and 11.6 respectively.

*Fungicides tested.* The range of fungicides tested were as follows: commercial formalin (38 per cent. formaldehyde); powders containing organically combined mercury (Abavit, Agrosan G, Ceresan (1875A), Lunasan, T.B. 910A); a soluble organo-mercurial preparation (Ceresan (U. 564)); and cuprous oxide powder manufactured according to the specifications laid down by Horsfall *et alia* (1934). Varying dosages of each fungicide were tested with each seed sample.

*Laboratory trials.* Tests made by the modified Ulster method.

*Field trials.* The experimental plots measured  $2 \times 2$  yds. and trials were made in duplicate at each of the two centres, the Agricultural Research Institute, Hillsborough, Co. Down, and The Farm, Stormont, Co. Down; a different seed sample was used for each experimental centre. At Hillsborough the crop was sown on May 8 and pulled on August 31; a severe outbreak of the disease, in all its phases, occurred in the control plots. Sowing was carried out at Stormont on May 6 and the crop was pulled on August 28; here a moderately severe attack of the seedling and stem-break phases of the disease occurred but the attack of browning was negligible.

#### 1940

*Seed sample.* In all experimental work for this year a seed sample of the variety Blue Star was used; 23.7 per cent. of the seeds of this sample were infected with *P. Lini*.

*Fungicides tested.* The fungicides tested included those used in 1939 with the exception of formalin and cuprous oxide. In addition the following were included: malachite green; a powder containing tetra-methyl thiuram disulphide as its active ingredient (RD. 7846); organo-mercurial powders adfixed to the seed with separated milk.

*Laboratory trials.* Tests made by the modified Ulster method.

*Field trials.* These were carried out in two series at the Agricultural Research Institute, Hillsborough, Co. Down.

*Series A.* The plots measured  $3 \times 3$  yds. and each treatment was replicated five times. Sowing was carried out on May 13-14 and the crop was pulled on August 22.

*Series B.* The plots measured  $2 \times 2$  yds. and each test was made in duplicate; they were sown on May 20 and pulled on August 23.

In both series severe attacks of all phases of the disease occurred in the control plots.

#### 1941

*Seed sample.* A single sample of the variety Stormont Gossamer was used containing 50.4 per cent. of seeds infected with *P. Lini*.

*Fungicides tested.* With the exception of malachite green and the addition



of further powders containing tetra-methyl thiuram disulphide (BS. 109, BS. 109A-C) the series of fungicides tested was essentially the same as in 1939.

*Laboratory trials.* Tests made by the modified Ulster method.

*Field trials.* These were carried out in three series at the Agricultural Research Institute, Hillsborough, Co. Down.

*Series A.* The plots measured  $4 \times 12$  yds. and the tests were made in quadruplicate. Sowing was carried out from May 7 to 13 and the crop was pulled from August 26 to September 1.

*Series B and C.* The plots measured  $2 \times 2$  yds. and the trials were made in duplicate. The seed was sown on May 6-7 (series B) and May 13 (series C). The crop was pulled on September 1 to 2.

Extremely severe attacks of all three phases of the disease occurred on the control plots of all three series. The attack of browning spread so rapidly that only one series of useful observations could be made.

#### 1940

*Seedling blight (C. Lini).*

*Seed samples.* Two seed samples were employed. For tests in series A a sample of the variety Liral Crown with 27.0 per cent. of the seeds infected with *C. Lini* was used, while for series B the sample was of the variety Stormont Gossamer with 35.7 per cent. of infected seeds.

*Fungicides tested.* The materials tested were the same as those used in the experiments dealing with the control of *P. Lini* in 1940.

*Laboratory trials.* Tests were made by the modified Ulster method.

*Field trials.* These were carried out in two series at the Agricultural Research Institute, Hillsborough, Co. Down.

*Series A.* The plots measured  $3 \times 3$  yds. and each treatment was replicated five times. The seed was sown on May 15 and the crop was pulled on August 24.

*Series B.* Each plot measured  $2 \times 2$  yds. and the tests were made in duplicate. Sowing was carried out on May 20 and the crop was pulled on August 24.

Severe attacks of the seedling phase of the disease occurred in all plots sown with untreated seed in both series of experiments.

#### 1941

*Seed sample.* One seed sample of the variety Liral Monarch was used containing 14.6 per cent. of seeds infected with *C. Lini*.

*Fungicides tested.* The same range of materials as were tested for the control of *P. Lini* in 1941 was employed.

*Laboratory trials.* Tests made by the modified Ulster method.

*Field trials.* These were carried out at the Agricultural Research Institute, Hillsborough, Co. Down.

*Series A.* Plots measuring  $4 \times 12$  yds. were used and the tests were made in

quadruplicate. The seed was sown on May 13 to 16 and the crop was pulled on September 1 to 2.

*Series B and C.* Each plot measured  $2 \times 2$  yds. and the tests were made in duplicate. The seed was sown on May 9 to 12 and the crop was pulled on September 2.

A moderately severe attack of seedling blight occurred in the control plots of all three series of trials.

#### COMPARISON OF RESULTS GIVEN BY LABORATORY AND FIELD METHODS

Data which has been accumulated over the three years during which this investigation has been in progress has allowed an accurate comparison to be made between the results obtained from laboratory methods and those given in the field. Detailed results from two series of experiments are given in Tables I and II. Those in Table I deal with trials carried out in 1939 for the prevention of stem-break and browning (*P. Lini*), while those in Table II are for a trial made in 1941 concerned with the prevention of seedling blight (*C. Lini*). Results in detail are available for all series of trials made during the experimental period, but in order to save space only two examples are given.

*Statistical analysis.* For seven of the series of experiments the percentage of seeds infected with *P. Lini*, as determined by the modified Ulster method, was correlated with both the seedling and stem-break phases of attack. In six of the series the values obtained by laboratory technique were correlated with the measure of the attack by the browning phase which had developed in the field on the first occasion when observations were made. In three trials, where a *number* of observations on the incidence of browning in the field plots were made during the season, the mean values obtained for treatments were correlated with the corresponding values obtained by the laboratory method. The coefficients of correlation obtained are given in Table III, and in order to indicate their significance the appropriate probability values (Fisher and Yates, 1938) are also shown. All the correlations are significant, six of the calculated values exceeding the 0.1 per cent. point, twelve exceeding the 1 per cent. point, three exceeding the 2 per cent. point, and two exceeding the requisite 5 per cent. point.

In the case of five series of experiments concerned with the prevention of seedling blight, it was possible to calculate the correlation between the percentage of seeds infected with *C. Lini*, as determined by the modified Ulster method, with the values obtained from observations on the severity of the seedling phase of attack in corresponding field tests. The correlation coefficients obtained are given in Table III together with the appropriate probability values. All the correlations are highly significant, two of the values calculated exceeding the 0.1 per cent., two exceeding the 1 per cent. point, and one exceeding the 2 per cent. value.

TABLE I

*Trials with Seed Disinfectants for the Prevention of Stem-break  
and Browning (P. Lini), 1939*

Seed sample used: Stormont Circus (1938) A.

Field trials carried out at the Agricultural Research Institute, Hillsborough, Co. Down.

Plots (in duplicate): 2 x 2 yds. Field observations recorded as the calculated mean for each pair of plots receiving similar treatment.

A-F = proprietary materials.

Material tested.	Method and rate of application per kg. of seed.	Laboratory method (500 seeds examined) per cent. infected seeds.	Field method Maximum attack by <i>P. Lini</i> = 10.		
			Seedling phase.	Stem-break.	Browning.
Untreated	—	11.6	10	10	10
Formalin 1 in 50	Short wet, 40 c.c.	9.4	6	0	10
" " " 80 c.c.	"	8.2	0	0	5
" 1 in 60	" 40 c.c.	9.8	3	3	10
" " " 80 c.c.	"	4.8	2	0	5
" 1 in 70	" 40 c.c.	8.6	3	0	8
" " " 80 c.c.	"	7.4	0	0	6
" 1 in 80	" 40 c.c.	10.6	5	5	8
" " " 80 c.c.	"	7.4	2	0	5
Cuprous oxide	Dry, 3 gm.	10.8	6	5	8
" " " 6 gm.	"	12.0	2	4	7
A	" 3 gm.	7.6	6	4	8
A	" 6 gm.	6.2	0	2	7
A	" 12 gm.	5.8	0	0	6
B	" 3 gm.	5.0	5	0	9
B	" 6 gm.	4.8	0	2	9
B	" 12 gm.	2.4	0	1	5
C	" 3 gm.	5.0	0	2	8
C	" 6 gm.	2.8	0	1	8
C	" 12 gm.	3.4	0	0	3
D	" 3 gm.	9.6	4	0	10
D	" 6 gm.	3.2	4	2	8
D	" 12 gm.	0.8	0	0	6
E	" 3 gm.	8.6	8	4	9
E	" 6 gm.	8.0	5	2	8
E	" 12 gm.	5.8	1	2	7
F	Short				
F	2% wet, 40 c.c.	11.2	2	0	8
F	" 80 c.c.	9.0	0	0	5
F	4% " 40 c.c.	7.4	2	0	4
F	" 80 c.c.	3.4	0	0	3
F	8% " 40 c.c.	5.4	0	0	4
F	" 80 c.c.	0.8	0	0	2

The results of the statistical analysis show that the evaluation of seed disinfectants in the laboratory for the prevention of either *P. Lini* or *C. Lini*,

using the modified Ulster method, may be regarded as very satisfactory in that the results so obtained are highly correlated with those given by corresponding trials in the field.

TABLE II

*Trials with Seed Disinfectants for the Prevention of Seedling  
Blight (C. Lini), 1941. Test C*

Seed sample used: Liral Monarch (1940).

Field trials carried out at the Agricultural Research Institute, Hillsborough, Co. Down.

Plots (in duplicate):  $2 \times 2$  yds. Field observation recorded as the calculated mean for each pair of plots receiving similar treatment.

G-K = proprietary materials.

Material tested.	Method and rate of application per kg. of seed.	Laboratory method (500 seeds examined) per cent. infected seeds.	Field method Maximum attack by <i>C. Lini</i> = 10.
Untreated	—	14.6	10
G	Dry, 3.0 gm.	0	2
G	" 6.7 "	0	0
G	" 12.0 "	0.6	0
H	" 3.0 "	0	0
H	" 6.7 "	0.8	0
H	" 12.0 "	1.8	0
I	" 3.0 "	1.2	0
I	" 6.7 "	1.0	2
I	" 12.0 "	0.2	0
J	" 3.0 "	0.4	0
J	" 6.7 "	0.4	0
J	" 12.0 "	0.2	0
K	" 3.0 "	0	0
K	" 6.7 "	0	0
K	" 12.0 "	0.2	0

TABLE III

*Correlation Coefficients. The Prevention of Stem-break and Browning (P. Lini)*

Year	<i>n</i>	Laboratory method <i>v.</i> seedling phase in field test.	Laboratory method <i>v.</i> stem-break phase in field test.	Laboratory method <i>v.</i> browning in field test (first observation).	Laboratory method <i>v.</i> browning in field test (mean of all observations).
1939	30	+0.5532	+0.5153	—	—
1939	30	+0.5695	+0.4779	+0.4531	+0.5209
1940 Test A	15	+0.8074	+0.7023	+0.6626	+0.7596
1940 Test B	25	+0.6356	+0.5639	+0.5928	+0.6898
1941 Test A	9	+0.8292	+0.7138	+0.7511	—
1941 Test B	28	+0.4629	+0.4329	+0.4028	—
1941 Test C	14	+0.8373	+0.6054	+0.5455	—



The Prevention of Seedling Blight (*C. Lini*)

Year	n	Laboratory method v. seedling phase in field test.
1940 Test A	15	+0.6284
1940 Test B	24	+0.4534
1941 Test A	9	+0.8908
1941 Test B	28	+0.5061
1941 Test C	14	+0.9549

## Values of the Correlation Coefficient for Different Levels of Significance

n	P = 0.05	P = 0.02	P = 0.01	P = 0.001
9	—	0.6851	0.7348	0.8471
14	0.4973	0.5742	0.6226	0.7420
15	—	—	0.6055	0.7246
24	0.387	0.451	0.494	0.606
25	—	—	0.4869	0.5974
28	0.359	0.421	0.461	0.568
30	—	0.4093	0.4487	0.5541

$n$  = number of treatments compared, less 2.

## SUMMARY

1. An account is given of laboratory and field methods devised for the evaluation of seed disinfectants used for the prevention of seed-borne diseases of flax. *Polyspora Lini* Laff. the cause of stem-break and browning and *Colletotrichum Lini* (Westerd. Toch.) the cause of seedling blight were the parasites specially considered. The laboratory method which is based upon the Ulster method used for the examination of flax seed samples for the presence of seed-borne parasites, can be carried out within a period of seven days at any time of the year.

2. The statistical analysis of the results shows a high correlation between those given by the laboratory method and those obtained in the field, so that the evaluation of seed disinfectants in the laboratory may be regarded as reliable and accurate.

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# Further Cytogenetical Investigations in the Genus *Gaura*

BY

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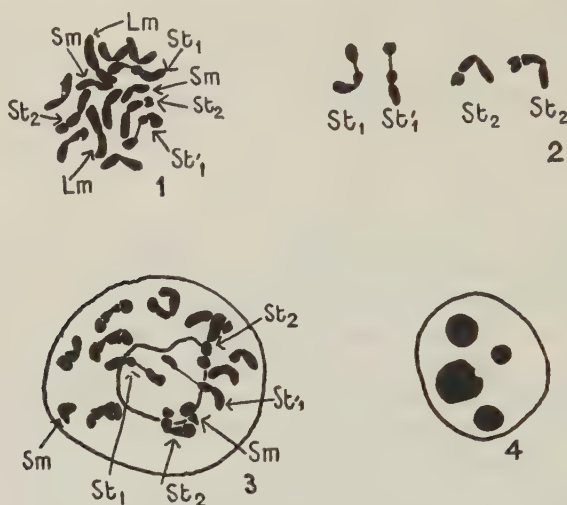
**With Plate VI and thirty-three Figures in the Text**

## INTRODUCTION

IN a previous communication (Bhaduri, 1941a) a cytological account was presented of a plant identified as *Gaura Lindheimeri*. From evidences obtained at that time it was concluded that the plant under investigation was a mutation. It was revealed then also that there is a close phylogenetic relationship between the two genera *Gaura* and *Oenothera*. It was shown in the above paper how a cytological analysis of the chromosome-nucleolus relationship may yield valuable results in tracing the evolutionary history of the nucleus. A new method of tracing the homology of the nucleoli in a species from an analysis of the relative size-difference between nucleoli and their segregation during meiosis was also described in the paper. Recent observations of a number of cases have shown (Bhaduri, in the press) that segmental interchanges between nucleolar and non-nucleolar chromosomes may alter the number and morphology of both nucleoli and sat-chromosomes. On the basis of the information referred to above, and taking into consideration on the one hand that no critical cytological or genetical observations have yet been made in the genus *Gaura*, excepting the one mentioned above, and on the other hand that species of *Gaura* show marked evidence of extensive segmental interchanges between chromosomes, it was thought that a comprehensive cytogenetical observation in different species of *Gaura* should not only throw considerable light on problems pertaining to the relation of chromosomes to nucleoli, but will also help considerably in the attempt to follow the course of evolution in the genus *Gaura*. It was realized also that a cytological re-examination of a number of plants of *G. Lindheimeri* from different sources should be made in order to verify the mutant nature of the plant. During the summers of 1940-1 a number of plants of *G. Lindheimeri* and *G. biennis* were therefore raised from seed obtained from Kew Gardens, seed of these two species only being available in this country. Cytological observations of these two species on the lines mentioned above brought out interesting and fruitful results and constitute the principal matter of the present paper.

## MATERIAL AND METHODS

Plants were raised in the Botanical Garden of the University of Bristol from the seeds obtained from Kew. Flower buds collected from healthy plants were pretreated with Carnoy's solution and fixed in medium Flemming's solution. Root tips were collected from fresh adventitious roots of young



TEXT-FIGS. 1-4. Fig. 1. Somatic complement of chromosomes of *G. biennis*. Fig. 2. The two pairs of nucleolar chromosomes drawn separately. Fig. 3. Somatic prophase showing the attachment of the four nucleolar chromosomes by their secondary constrictions to the fused nucleolus. The ten other chromosomes lie free in the nucleus. Fig. 4. Somatic nucleus with four nucleoli of three different sizes. ( $\times 2,800$ .)

plants grown in sand (Bhaduri, 1940, 1941a). They were fixed in Lewitsky's chromic-formalin mixtures (1:1 and 6:4). Paraffin sections, 20 to 22  $\mu$  thick for flower buds and 12  $\mu$  thick for root tips, were made. These were stained both in gentian violet-iodine and fuchsin-light green combinations. Smear preparations of root tips, pollen mother-cells, and pollen grains were made following the methods previously described by the author (Bhaduri, 1941).

Drawings were made with a Zeiss 2 mm. apochromatic objective 1.4 N.A. with a homogeneous immersion aplanatic condenser 1.3 N.A. and compensating eyepieces  $\times 10$  and  $\times 20$ .

## OBSERVATIONS

*Gaura biennis* L. ( $2n = 14$ ).

*Somatic.* Like *G. Lindheimeri* and *G. coccinea*, *G. biennis* has fourteen chromosomes in the root tip cells. The chromosomes are very small, 2.8  $\mu$  to 1.8  $\mu$ , and show only slight size-differences. They could be classified, however, into groups according to their sizes and the relative positions of the primary and secondary constrictions. There are two long and two small



chromosomes with median primary constrictions (Lm and Sm) and six intermediate-sized chromosomes slightly varying in sizes with nearly median primary constrictions (Im). Besides the ten chromosomes of the above types there are four more chromosomes with a marked secondary constriction in each. These four chromosomes with secondary constrictions, the four satellited chromosomes, form two distinct pairs,  $St_1 St'_1$  and  $St_2 St_2$  (Text-fig. 1). One pair, the  $St_1 St'_1$ , consist of two small chromosomes with subterminal primary constrictions, each having a disproportionately large secondary constriction.

In each of these two chromosomes the small segment of the chromosome or the satellite-head, separated from the body of the chromosome by the secondary constriction, is very large and conspicuous. This pair of chromosomes are heteromorphic with respect to the size of the satellite-heads and the secondary constrictions, one being slightly larger than the other (Text-fig. 2). Similar observations have been recorded before in *G. Lindheimeri* and some *Oenothera* species (Bhaduri, 1940, 1941a). The second pair of sat-chromosomes, i.e.  $St_2 St_2$ , are longer than the first pair and have slightly submedian primary constriction. The secondary constrictions in this pair are not large as in the first pair, though they are well marked and the satellite-heads are quite conspicuous (Text-fig. 2). As in all other plants both the satellite-head and the filament were found to be Feulgen-positive and were picked out clearly, following the fuchsin-light-green staining, as bright magenta-coloured bodies against the green background of the nucleolus.

Corresponding to the four secondary constrictions the maximum number of nucleoli in the somatic nuclei was found to be four. It may be concluded therefore that all the four secondary constrictions are nucleolar. During prophase the four nucleolar chromosomes were found invariably attached to the fused nucleolus by their secondary constrictions, while the remaining ten other chromosomes were seen lying free in the nucleus. In favourable preparations most of the chromosomes could be identified at this stage (Text-fig. 3). As previously described in heterozygous species of *Oenothera* and in *G. Lindheimeri* (Bhaduri, 1941a), the four nucleoli of a somatic nucleus were found to be of three different sizes, one large, one small, and two intermediates (Text-fig. 4). This relative size-difference between nucleoli was found to be distinct and constant. From a careful analysis of the sizes of the nucleoli and their segregation during meiosis, by a method which will be described later in this paper, it was established that the four nucleoli form two heteromorphic pairs; the large with one intermediate and the small with the other intermediate constitute the two heteromorphic pairs.

The resting nuclei of the root tip cells always showed the presence of heterochromatic portions of the chromosomes; their number generally corresponded with the diploid number of the chromosomes.

*Meiotic.* The morphology and the development of the sporogenous tissue in the anther was found to be as described before in the case of the mutant plant

of *G. Lindheimeri* (Bhaduri, 1941a). The pollen mother-cells during early stages of meiosis remain closely packed in the anther loculus, but separate later from the surrounding tapetal cells and are held in the form of a loose string. Preparations so far obtained did not allow critical study of the nature



TEXT-FIGS. 5-13. Fig. 5. Zygotene: three nucleoli, one very small. Note the nucleolar body on the big nucleolus (fuchsin-light-green). Fig. 6. Attachment of two pairs of chromosomes to the nucleolus. Fig. 7. One of the chromosomes attached to the nucleolus showing a marked secondary constriction. Fig. 8. Maximum catenation of  $(12) + I_{II}$ ; note the position of the pair of long chromosomes in the ring. Fig. 9. The ring of twelve chromosomes attached to the nucleolus. Figs. 10-13. Showing various kinds of catenations, attachment of chromosomes to nucleoli, and interlocking of the bivalent. ( $\times 2,800$ .)

of the pairing of chromatids, only from diplotene onwards could the nature of the association of the chromosomes be followed. During diakinesis a distinct catenation of the chromosomes was observed. The maximum catenation for this species was found to be a ring of twelve chromosomes and one bivalent (Text-figs. 8, 9, 33; Pl. VI, fig. 1). Instead of a ring of twelve, one,

two, or more chains consisting of varying numbers of chromosomes were observed (Text-figs. 10, 11, 12, 13). The pair of long chromosomes with median primary constriction found in the somatic complement could, in favourable spots, be identified lying end to end in the ring (Text-fig. 8); the pair of small chromosomes were also found included in the ring. The end-to-end orientation of the homologous chromosomes in the ring is in conformity with the theoretical expectations demanded by the segmental interchange theory for ring formation in *Oenothera* (Hakansson, 1928, 1930; Darlington, 1929, 1931). The bivalent chromosome was generally found lying apart from the ring, though sometimes it was observed interlocked in the ring (Text-figs. 8, 11, 13). Two of the intermediate-sized chromosomes of the somatic complement constitute the bivalent.

During early stages of meiosis pollen mother-cells frequently showed the presence of two nucleoli in a nucleus, one being much larger than the other. Sometimes three, one quite large, one intermediate, and another very small nucleoli, were found (Text-fig. 5). In later stages only one large nucleolus, the fusion product of the four nucleoli, was generally observed. The ring of twelve chromosomes was generally found attached to the nucleolus (Text-figs. 9, 11; Pl. VI, Fig. 2). The bivalent chromosome, on the contrary, was always seen lying free from the nucleoli. Evidently the ring includes all the four nucleolar chromosomes. In favourable spots it was found that the ring was attached to the nucleolus at two widely separated points (Text-figs. 9, 12), which shows that the two pairs of nucleolar chromosomes do not lie consecutively in the ring. During early diplotene stage two pairs of chromosomes were also found attached separately to the fused nucleolus (Text-fig. 6). Although secondary constrictions of chromosomes were not generally clear at these stages, yet cases showing definitely the attachment of the chromosomes to the nucleolus by secondary constrictions have been observed (Text-figs. 7, 12).

During metaphase the ring of twelve chromosomes orientate in the usual zigzag manner as characteristic of 'complex-heterozygotes', so that paternal and maternal chromosomes may pass to opposite poles. Though the orientation of the ring was found to be fairly regular, irregular segregation leading to double non-disjunction on the same or opposite sides was observed not infrequently (Text-figs. 15, 16). Double non-disjunction on both sides produce genetically unbalanced pollen grains with normal haploid number. Such double non-disjunctions could only be detected during early stages of first anaphase. Though the chromosomes were found to separate regularly, some irregularities with reference to the timing of the separation of the homologues were frequently observed. Some pairs were seen to separate much earlier than others; sometimes it was found for instance that while one or two chromosomes have already reached the poles the others were still lying at the equatorial region, some of them not yet separated even from their respective homologues (Text-figs. 14, 15). Similar observations have



also been recorded previously in case of *G. Lindheimeri* (Bhaduri, 1941a). Due to non-disjunction of chromosomes a distribution of 8 and 6 chromosomes to two opposite poles was frequently observed (Text-fig 17). No secondary association of chromosomes was noticed either during the first or second divisions. The usual distribution was found to be one in the centre surrounded by six others at the periphery (Text-fig. 18). Relative size-difference between the chromosomes could also be detected during these stages (Text-figs. 17, 18). Both the second division and tetrad formation were found to be normal without showing any irregularities.

Excepting when they are fused, either one big and one small or two medium-sized nucleoli were found, in equal proportion, in most of the microspores from tetrad stage to well-formed pollen grains (Text-figs. 19, 20). Rarely, however, three, one big and two intermediate-sized, nucleoli were seen in the nucleus of a microspore (Text-fig. 21). This latter condition was evidently due to non-disjunction involving the nucleolar chromosomes. As we know that all the four nucleolar chromosomes are included in the ring and that alternate chromosomes pass to opposite poles, the presence of two intermediate-sized nucleoli in the nuclei of nearly 50 per cent. of the microspores clearly indicate that the large with one intermediate-sized nucleolus and the small with the other intermediate-sized constitute the two heteromorphic pairs (cf. Bhaduri, 1941a). This observation is in agreement with the previous observation recorded in the case of the mutant plant of *G. Lindheimeri* and confirms once again the view that relative size-difference between nucleoli is a constant character of a species.

The mature pollen grains of *G. biennis* are triangular in shape with three lobes placed at the three corners of the grain, this is also characteristic of the pollen grains of species of *Oenothera*. In *Gaura* the outer wall of these lobes showed the presence of alternate rows of specially thickened and thin bands. The inner wall of the pollen grains was also found highly thickened. In both *G. biennis* and *G. Lindheimeri* it was found that the first microspore division is completed before shedding (Text-figs. 29, 31, 33).

The *nucleolar body*, found generally in the pollen mother-cells of *Oenothera* and also described before in the case of *G. Lindheimeri*, was frequently observed in the pollen mother-cells of *G. biennis* (Fig. 5). With the fuchsin-light-green staining these bodies took up the deep magenta colour of the fuchsin and were sharply differentiated against the green background of the nucleolus. From the morphology and staining reaction of these bodies it was concluded that they are specialized heterochromatic portions of sat-chromosomes responsible for the formation of the nucleolus. Recent observations in some species of *Scilla* have clearly shown that nucleoli are organized around such heterochromatic portions of the sat-chromosomes (Bhaduri, in the press). These specialized heterochromatic portions of sat-chromosomes are distinguishable from the rest of the body of the chromosome only at certain stages of the nuclear cycle. While in the case of *Oenothera* and

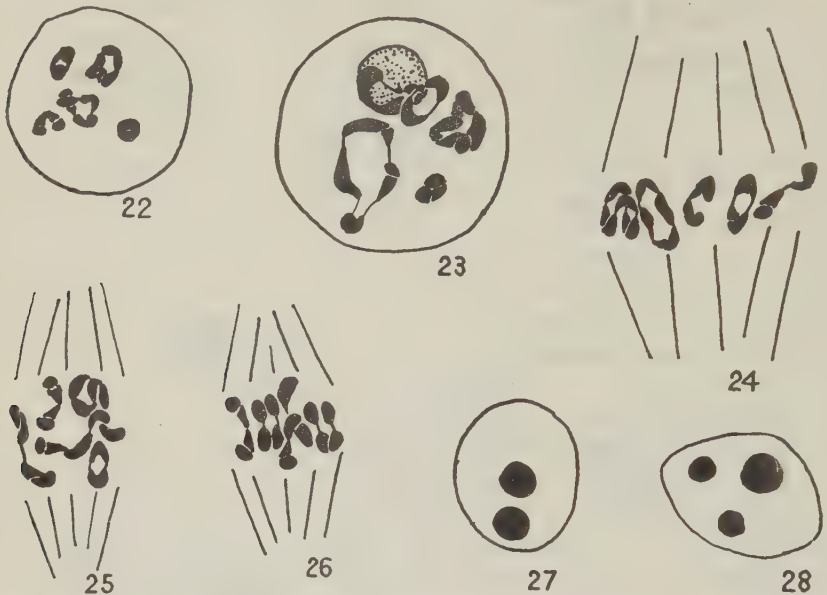




TEXT-FIGS. 14-21. Figs. 14-16. Showing the manner of separation of the chromosomes during first anaphase. Note double non-disjunctions in Figs. 15 and 16. Fig. 17. Note 8 and 6 distribution due to non-disjunction. Fig. 18. Polar view of first anaphase showing six chromosomes on the periphery and one in the centre. Note the size-difference of the chromosomes in Figs. 17 and 18. Figs. 19 and 20. Showing nucleoli in microspores at tetrad stage. Fig. 19. The big and the intermediate nucleoli. Fig. 20. The two intermediate-sized nucleoli. Fig. 21. A microspore nucleus with three nucleoli due to non-disjunction. ( $\times 2,800$ .)

*Gaura* they were distinguishable during earlier stages of meiosis, in the case of certain species of *Scilla*, on the other hand, it was noticed that this is so only during very early prophase of the first microspore division (Bhaduri, in the press). According to Schultz (1939, 1941) the heterochromatic portions

of chromosomes in the salivary gland of *Drosophilla* are responsible for the synthesis of ribo-nucleic acid which is one of the chief chemical components of the nucleolus. It is interesting to point out here that Sikka (1940) in case of an interspecific hybrid of *Oenothera* did not find these bodies stained



TEXT-FIGS. 22-8. Fig. 22. Maximum catenation in *G. Lindheimeri* Engelm & Gray.  $2(4) + 3n$ . Fig. 23. Two bivalent pairs attached to the fused nucleolus, the two rings of four and the remaining bivalent lying free in the nucleus. Fig. 24. Orientation of the chromosomes during first anaphase. Figs. 25 and 26. First anaphase; note the orientation of some of the chromosomes indicating double non-disjunction. Fig. 27. A microspore nucleus with two nucleoli of same size. Fig. 28. Same with three nucleoli due to non-disjunction, the big and the two intermediates. ( $\times 2,800$ .)

with Feulgen. He concluded therefore that the nucleolar body is non-chromatic.

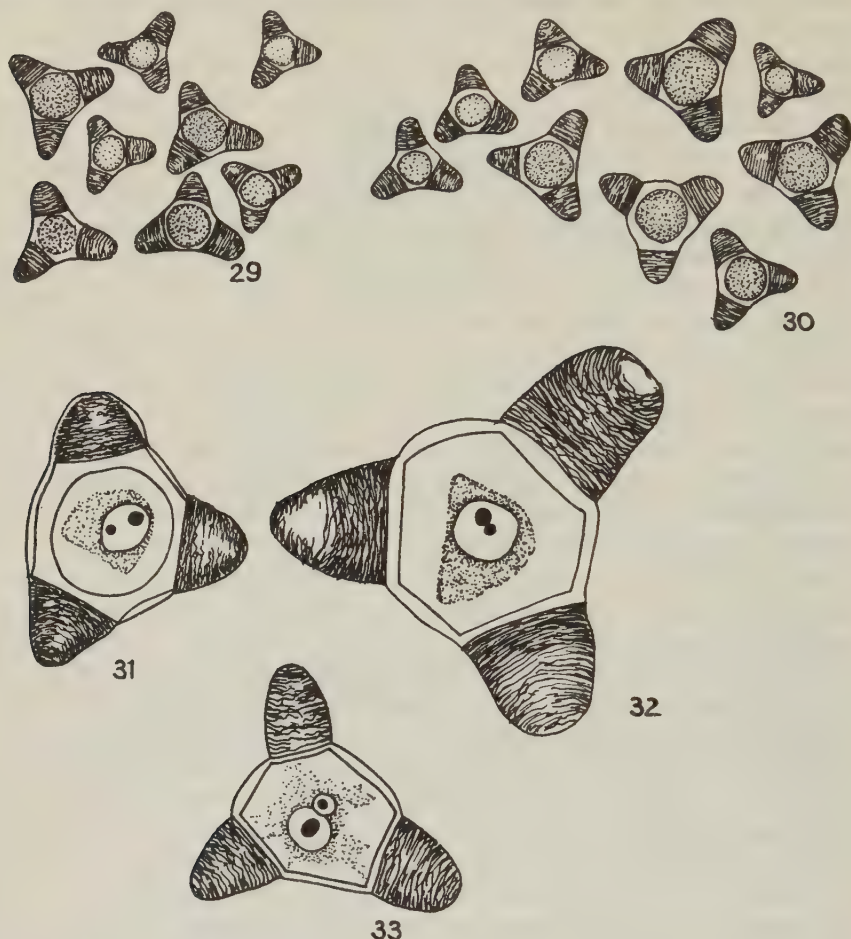
#### *Gaura Lindheimeri* L. ( $2n = 14$ )

It was found that the plants raised from seeds of *G. Lindheimeri* show marked difference in cytological characters from the plant previously described as a mutation (Bhaduri, 1941a). It is proposed therefore to describe here the cytological characters of *G. Lindheimeri* Engelm & Gray, especially bringing out those points by which it differs from the mutant plant. The morphology of the somatic chromosomes and the chromosome-nucleolus relationship in *G. Lindheimeri* Engelm & Gray have been described in a previous paper (Bhaduri, 1941a) and need not be repeated here. It is worth pointing out, however, that the idiograms of the two species *G. biennis* and *G. Lindheimeri* are similar.

The maximum catenation of *G. Lindheimeri* was found to be two rings of four and three bivalents, instead of one ring of six and four bivalents as found in the mutant plant (Text-fig. 22; Pl. VI, Fig. 2). Out of the three bivalents two were constantly found attached to the fused nucleolus. The two rings of four on the other hand were mostly found lying free from the nucleolus (Text-fig. 23). It may be concluded, therefore, that the two bivalents found attached to a nucleolus contain the two pairs of nucleolar chromosomes present in the somatic complement of this species. Although one quite large and another small nucleolus were frequently observed in the nuclei of the pollen mother-cells, generally all the four nucleoli of the somatic nuclei were found to fuse with each other forming a big nucleolus. Pollen analysis of this species showed that pollen grains have either one big and another small or two medium-sized nucleoli in their nuclei (Text-figs. 27, 32). The proportion of these two types of grains in the anthers were found to be nearly equal. Considering the above observation on the one hand and on the other the fact that the four nucleolar chromosomes form two pairs, it may be concluded that the large and one intermediate-sized, and the small with the other intermediate-sized nucleolus constitute the two heteromorphic pairs corresponding to the two heteromorphic pairs of sat-chromosomes. Conditions of sat-chromosomes and nucleoli in *G. Lindheimeri* are therefore identical with those found in the case of *G. biennis*. The orientation of the two rings of four chromosomes during metaphase was found to be regular whereby alternate chromosomes in the ring were seen passing to opposite poles (Text-figs. 24, 25; Pl. VI, Fig. 3). Double non-disjunction on the same or opposite side was observed not infrequently (Text-fig. 26). As described before in case of *G. biennis*, the separations of the homologous chromosomes were not synchronized in relation to time; some pairs were found separating earlier than others. That this delay in separation was due to some kind of mechanical interference was indicated by the appearance of chromatic bridges or attenuations between two homologous chromosomes. Similar observations have been recorded before in case of *G. biennis* (Text-fig. 14) and some species of *Oenothera*. According to Catchside (1932) this peculiar appearance is due to incomplete terminalization of chiasmata. The second meiotic division and tetrad formation were found to be regular and normal. Abnormalities in tetrad formation leading to the formation of giant grains and other irregularities commonly met with in the case of the mutant plant were not noticed in the case of these plants raised from seeds of *G. Lindheimeri* Engelm & Gray.

It was found in the case of both *G. biennis* and *G. Lindheimeri* that the mature pollen grains are of two distinct types, the larger type being filled with dark cell contents, whereas the smaller type appears more or less empty (Text-figs. 29, 30). Nearly 60 per cent. of the pollen grains were found to be of the smaller empty type. It was found also that the pollen grains of *G. Lindheimeri* were much larger than those of *G. biennis* (Text-figs. 31, 32). Whether

there is a correlation between the degree of catenation of chromosomes and different size of pollen grains needs to be determined by observations in other species of *Gaura*. The difference in the sizes of the pollen grains may,



TEXT-FIGS. 29-33. Figs. 29 and 30. Two types of pollen grains in *G. biennis* and *G. Lindheimeri* respectively. ( $\times 150$ .) Figs. 31 and 32. Magnified view of mature pollen grains of *G. biennis* and *G. Lindheimeri* respectively. Note the number and size-difference of nucleoli in each nucleus. ( $\times 1,100$ .) Fig. 33. A fully mature pollen grain of *G. Lindheimeri* with the vegetative and the generative nuclei. ( $\times 520$ .)

however, be profitably used as an additional distinguishing character between the two species, *G. biennis* and *G. Lindheimeri*.

#### DISCUSSION

It has been pointed out previously that there is a close phylogenetic relationship between *Gaura* and *Oenothera*. It is now well known that the



genetical behaviour of most of the species of *Oenothera* is, in certain respects, sharply distinguishable. The species of *Oenothera* have fourteen chromosomes and theoretically should, therefore, have seven independent linkage groups; many, however, behave as though they had but a single group. With respect to this one group, they are highly heterozygous and produce gametes of only two kinds from the standpoint of the 'gene complexes' which they carry. These species are 'complex heterozygotes' and breed true when selfed, due to the operation of a balanced lethal system. The heterozygosity of these species is, however, expressed in outcrosses. Thus those species which produce two kinds of functional eggs (e.g. *O. Lamarckiana* or *O. grandiflora*) tend to produce twin or multiple types in their progenies; those species, on the other hand, which produce but one kind of functional sperm, or one kind of functional egg (e.g. *O. muricata*), produce unlike reciprocals. It may be said in short that the peculiar genetical feature of most of the *Oenothera* species is associated with three principal phenomena, namely, (1) balanced lethal system, (2) ring formation or catenation of chromosomes during meiosis, (3) association of many genetical factors into one linkage group.

The balanced lethal system may be of two types. The first type prevents the development or functioning of those eggs or sperms which carry a given set or complex of genes; these are called *gametic lethals*. For example Renner (1919) found in *O. muricata* two types of pollen grains in almost equal proportions, one *active* (*curvans*, carrying grains), filled with smaller blunt-ended starch grains, the other *inactive* (*rigens*, carrying grains) with longer spindle-shaped starch grains. The second type, the so-called *zygote lethals*, prevent the development of individuals which have received the same complex from both parents. Although breeding experiments have not yet been made in case of *Gaura* species it appears that both *G. biennis* and *G. Lindheimeri* belong to the gametic, lethal type, the distinction between the two types of pollen grains in case of *Gaura* species being sharper than found in *O. muricata*. The active grains in both *G. biennis* and *G. Lindheimeri* were found to be larger and filled with dark cell contents, whereas the inactive grains were smaller and appeared more or less empty. The increase in the percentage of the inactive grains, i.e. 60 per cent., instead of the 50 per cent. to be theoretically expected, is to be accounted as due to the intervention of another factor, namely non-disjunction of some of the chromosomes during meiosis.

It is well known now that the fourteen chromosomes in most of the species and hybrids of *Oenothera* instead of forming seven bivalents during diakinesis may form a complete ring of fourteen chromosomes or two or three rings of chromosomes involving a smaller number of chromosomes, or one or more rings together with one or more bivalents. The maximum number of chromosomes in a ring or the maximum numbers of rings and bivalents in a species is always constant. Another characteristic feature of meiosis in *Oenothera* is the zigzag orientation of the ring of chromosomes during first metaphase,

whereby alternate chromosomes of the ring pass to opposite poles. The original assumption that paternal and maternal chromosomes alternate in the ring and that by this unique way of orientation the paternal chromosomes go to one pole and the maternal to the other has been amply verified by breeding experiments. The segmental interchange theory originally propounded by Belling (1927) has fairly explained the origin of ring formation by chromosomes during diakinesis. According to the segmental interchange theory as applied to ring-forming *Oenothera*s the two end segments of a chromosome in a ring are homologous with the two different end segments of two different chromosomes placed at either end of the first one. In other words each chromosome in a ring has at least two non-homologous end segments. The above conception regarding the structure and genetic constitution of chromosomes provides, on the one hand, an indication of the physical basis of the extensive genetic linkage present in *Oenothera* species, and on the other, a clue to the problem as to how a single balanced lethal system may control a large number of linkage groups. That each gene complex of a species of *Oenothera* has its own specific arrangement of end segments is now well known. There are fifteen different possible ways of arrangement or catenation of the fourteen chromosomes. All these fifteen different types of catenation have been observed in different species and hybrids of *Oenothera*.

All the above cytological peculiarities associated with the characteristic genetical behaviour of *Oenothera*s have been observed also in the species of *Gaura*. Although breeding experiments with different species of *Gaura* have not yet been made, the following principal observations—the formation of two kinds of pollen in *G. biennis* and *G. Lindheimeri*, the high degree of linkage of chromosomes in *G. biennis*, and the fact that both *G. biennis* and *G. Lindheimeri* breed true when selfed—clearly show that these two species of *Gaura* are complex heterozygotes.

From the list of catenations compiled by Gates and Ford (1938) it was noticed that according to an unpublished report of Burkert the maximum catenations of *G. biennis* and *G. Lindheimeri* are (14) and 7<sub>II</sub> respectively. It has been shown clearly during the present observation, however, that the maximum catenations of the two species are (12) + 1<sub>II</sub> and 2 (4) + 3<sub>II</sub> respectively. It is feared therefore that Burkert may have made some mistake either in the determination of the maximum catenation or in the identification of the species. It has been pointed out above that all the fifteen possible catenations have been recorded for different species and hybrids of *Oenothera*. In the case of *Gaura* only three different types have been so far observed, namely, (12) + 1<sub>I</sub> in *G. biennis*, 2 (4) + 3<sub>II</sub> in *G. Lindheimeri*, and (6) + 4<sub>II</sub> in a different form of *G. Lindheimeri*. Examination of other species and races of *Gaura* will certainly show the existence of the other types of catenations. It was pointed out in the previous account (Bhaduri, 1941a) that the maximum catenation of (6) + 4<sub>II</sub> found in a different form of

*G. Lindheimeri* was very significant in the sense that this catenation has not been recorded for any naturally-occurring species of *Oenothera*. The same conclusion holds good for the other catenation, i.e.  $2(4) + 3_{II}$  found in *G. Lindheimeri* Engelm & Gray. This particular catenation of  $2(4) + 3_{II}$  has, however, been recorded very frequently in a number of interspecific hybrids of *Oenothera* (cf. Gates and Ford, 1938).

It is now generally agreed that in the case of *Oenothera* the original large-flowered species with seven free pairs of chromosomes found in Central America and Mexico gave rise by a series of steps to species with small flowers and complete catenation now found widely distributed over the North American continent (cf. Gates, 1929). Genetical experiments have shown further that similarity in segmental arrangements of chromosomes indicates also phylogenetic relationships of species (cf. Cleland, 1936). These conclusions obtained after years of laborious research may very profitably be used in the genetical analysis of the species of *Gaura*. Compared with the very large number of species and races of *Oenothera* that of *Gaura* up to the present is very small (Munz, 1938); the distribution of the species of *Gaura* is also comparatively localized. It appears therefore that a comprehensive and thorough study in the cytogenetics of different species of *Gaura* is quite practicable. Such a study will certainly give valuable results throwing light on the correlation between chromosome catenation and the trend of evolution in the genus, and also provide an explanation of the principles underlying the parallel lines of evolution present in the genera *Oenothera* and *Gaura*.

The presence of four nucleoli, corresponding to the four chromosomes with secondary constrictions in the somatic nuclei of both *G. biennis* and *G. Lindheimeri*, confirms once again the theory of the numerical correlation between the number of nuclei and the number of sat-chromosomes present in a species (Heitz, 1931, 1931a). In a previous paper (Bhaduri, 1941a) it was pointed out that the presence of four nucleoli instead of two in the somatic nuclei of *G. Lindheimeri* might be either due to secondary polyploidy or to previous segmental interchanges between nucleolar and non-nucleolar chromosomes, as described by McClintock (1934) in the case of *Zea mays*. Recent observations in a number of widely different plant genera have led to the following general conclusion: *that diploid species may have more than two nucleoli in their somatic nuclei and that increase in the number of nucleoli in a species may take place not necessarily through polyploidy alone but also through structural changes of the chromosomes such as non-homologous segmental interchanges* (Bhaduri, in the press). In ring-forming species of *Oenothera* and *Gaura*, where evidence of secondary polyploidy is not yet known but where there is conclusive evidence of previous segmental interchanges, the increase in the number of nucleoli from two to four has to be interpreted as due to segmental interchanges between nucleolar and non-nucleolar chromosomes which must have taken place during the course of evolution. The difficulty still remains, however, of explaining the presence of four nucleoli in homozygous species



like *Oenothera Hookeri* on the one hand, which forms seven bivalents during diakinesis, and in heterozygous species like *O. Lamarckiana*, *O. ammophioides*, *O. biformiflora*, &c., on the other, which show a high degree of catenation (Bhaduri, 1940).

From observations so far recorded it may be assumed as fairly established that *the relative size-difference between nucleoli is a constant character of a species*. Further, *true diploid species must have a pair of homologous and identical nucleoli in their somatic nuclei*. *A change in the relative size-difference or homology of the nucleoli must be looked upon as a derived condition brought about by some sort of structural change of nucleolar chromosomes during the course of evolution*. Such structural changes may or may not be sufficiently well marked to induce a visible morphological change in the nucleolar chromosome. Both in *G. biennis* and *G. Lindheimeri* the heteromorphic nature of the nucleoli was apparent in both the pairs. In the case of the corresponding nucleolar chromosomes, however, it was found that while the heteromorphic nature was clearly distinguishable in one pair, in the other pair it was not at all distinct. While it is comparatively easy to trace the homology of the sat-chromosomes it is very difficult, especially where the chromosomes are small, to trace the homology of the nucleoli. A new method of tracing the homology of the nucleoli has been described elsewhere (Bhaduri, 1941a). From a study of the morphology and segregation of nucleoli during meiosis and in pollen grains of *G. biennis* and *G. Lindheimeri* it was found that out of the four nucleoli the large with an intermediate-sized and the small with the other intermediate-sized nucleolus constitute the two homologous pairs. This observation also confirms the previous observation made in a different form of *G. Lindheimeri*. The presence of heteromorphic pairs of nucleoli in *G. biennis* and *G. Lindheimeri* suggests at once not only the heterozygous nature of the two species, but also confirms the view that they are structural hybrids. In support of this conclusion the observation made in case of *Oenothera* species may be recalled. In the homozygous uncatenated species like *O. Hookeri*, only two distinct pairs of nucleoli were observed. In most of the heterozygous species, on the other hand, three different sizes of nucleoli, most probably constituting two heteromorphic pairs, were found (Bhaduri, 1940). Examination of nucleoli in some homozygous species of *Gaura* has still to be made; such an examination, it is expected, will substantiate the above conclusions. In view of the very important results already obtained it may be pointed out here that a study of the morphology of the nucleoli and their correlation with nucleolar chromosomes on the lines described above have great possibilities and may very profitably be used in cytological investigations.

#### SUMMARY

A cytogenetical investigation of *G. biennis* L. and *G. Lindheimeri* Engelm & Gray brought out the following important features. The fourteen chromosomes in the root tip cells of *G. biennis* could be classified into distinct groups



according to the then shape and size, the position of the primary constriction, and the presence or absence of a secondary constriction. Corresponding to the four nucleoli of three different sizes there were present in the somatic complement two heteromorphic pairs of chromosomes with a marked secondary constriction in each. Of the four nucleoli, the large with one intermediate-sized nucleolus and the small with the other intermediate-sized nucleolus constituted the two heteromorphic pairs. The homology of the nucleoli was established from an analysis of the number and relative size-difference between nucleoli in tetrads and pollen grains. The maximum catenation was found to be  $(12) + 1_{II}$  in the case of *G. biennis* L. and  $2(4) + 3_{II}$  in the case of *G. Lindheimeri* Engelm & Gray. This observation confirms the view that there are at least two different strains of *G. Lindheimeri* with different maximum catenations, namely  $(6) + 4_{II}$  and  $2(4) + 3_{II}$ . While in the case of *G. biennis* the four nucleolar chromosomes are included in the ring of twelve, in *G. Lindheimeri* on the other hand two out of the three bivalents constitute the two heteromorphic pairs. The idiograms of *G. biennis* L. and *G. Lindheimeri* Engelm & Gray were found to be identical. The mature pollen grains of *G. Lindheimeri* are bigger than those of *G. biennis* and can be used as an additional distinguishing character between the two species. The nucleolar body observed in the pollen mother-cells was found to be chromatic in nature. These bodies are most likely specialized heterochromatic regions of nucleolar chromosomes responsible for the organization of nucleoli.

In view of the observations made recently in a number of different genera as well as from the fact that there is no evidence of the presence of secondary polyploidy in either *G. biennis* or *G. Lindheimeri*, it has been concluded that the presence of four nucleoli instead of two in the somatic nuclei of these two species has to be interpreted as due to segmental interchanges between nucleolar and non-nucleolar chromosomes. The present observation provides further evidence for the view that the relative size-difference between nucleoli in a species is a constant character of the species. Difference in the sizes of nucleoli in a nucleus of a diploid species is to be looked upon as evidence of structural hybridity of the species brought about by structural changes of chromosomes.

The following principal observations, namely the production of two types of pollen grains, the high degree of chromosome catenation, and the fact that both the species breed true when selfed show that both *G. biennis* and *G. Lindheimeri* are complex-heterozygotes. Cytogenetical observations in *Gaura* not only revealed the close phylogenetic relationship between the genera *Oenothera* and *Gaura*, but also indicated the existence of parallel lines of evolution operating in the two genera.

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## EXPLANATION OF PLATE VI

Illustrating Dr. Bhaduri's article on 'Further Cytogenetical Investigations in the Genus *Gaura*'.

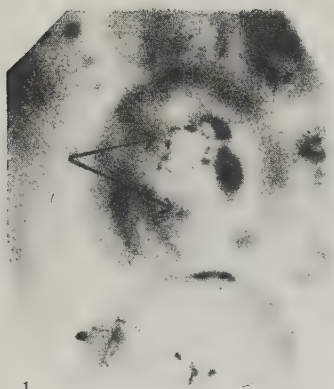
All figures are photomicrographs taken with the aid of a Zeiss's 'Phoku' apparatus. ( $\times 2,000$ .)

Fig. 1. Maximum catenation ( $12 + 1_n$ ) in *G. biennis* L.; compare with Text-fig. 8. The bivalent chromosome is lying far away from the ring and slightly out of focus.

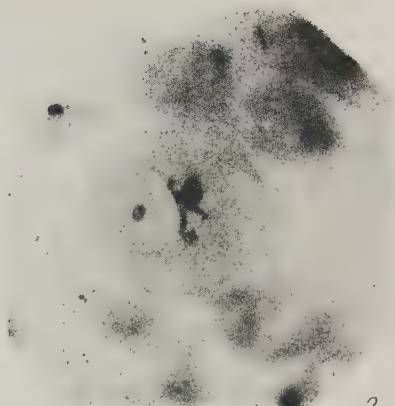
Fig. 2. The ring of twelve chromosomes attached to the nucleolus while the bivalent is lying free.

Fig. 3. Maximum catenation,  $2(4) + 3_n$ , in *G. Lindheimeri* Engelm & Gray. Compare with Text-fig. 22.

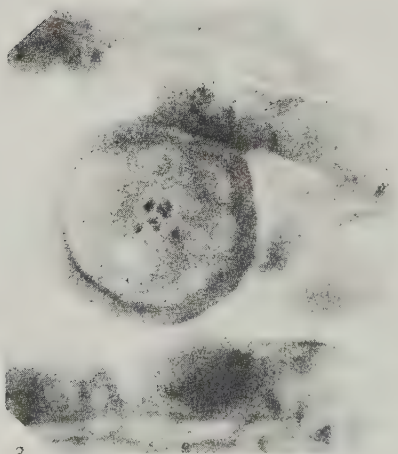
Fig. 4. Showing the orientation of  $2(4) + 3_n$  during 1st anaphase. Compare with Text-fig. 27.



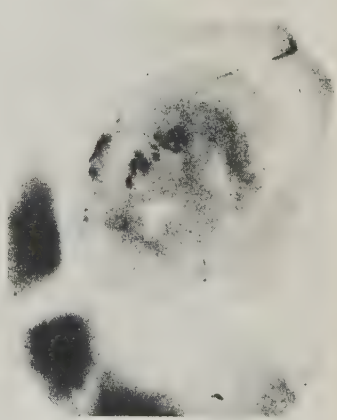
1



2



3



4

Huth, Stubbs X., Kent.

BHADURI — GAURA.





# Developmental Anatomy of the Shoot of *Zea mays* L.

BY

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With Plate VII and twenty-three Figures in the Text

## INTRODUCTION

IN the following account the development of a monocotyledon shoot is examined in relation to the probable movement of food and water in the plant during different growth phases. In the course of the work various points emerge as to development, structure, and morphology, of which the significance is discussed after the observations have been placed on record.

## MATERIALS AND METHODS

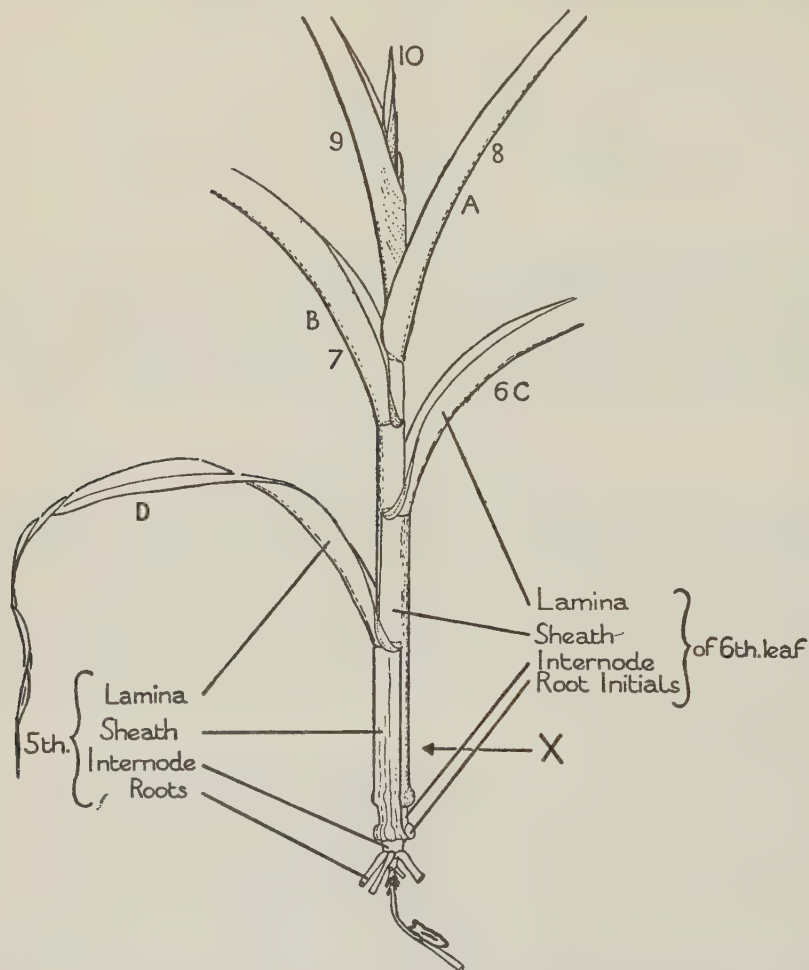
Seeds of Sutton's White Horse Tooth maize were planted singly in 3-in. pots in an unheated greenhouse. The seedlings were gradually advanced to 6-in. pots, plants being selected and preserved at various stages until about the 12th leaf from the coleoptile was exposed as far as the ligule. The remaining plants were transplanted into open ground to provide material for observations on more mature specimens. An additional crop was planted directly into the deeply dug bed of an unheated greenhouse and samples were taken throughout the growing period.

Material for the examination of apices was at first fixed in chrom-acetic acid, but better results were obtained with formalin-alcohol: neither fixative, however, gave results which were as good as could be desired. Attempts to use neutral fixatives (e.g. neutral 4 per cent. formaldehyde in aqueous solution) to preserve details of the vacuome of the apical cells gave little success with *Zea*, although similar fixatives had been found ideal for the perennial herbage grasses. In *Zea* the cytoplasm seems particularly difficult to fix satisfactorily. Sections were originally stained in safranin and fast green, but the tannic acid and iron staining of Kliem (1931) was found superior for the finer details of the apical meristematic cells.

Whole plants were also pickled in a long tank in a formalin-glycerine-alcohol mixture designed to plasmolyse the cell contents before fixation. This material was found particularly useful for comparison with observations made on 'microtomed' material: the plasmolysed cell contents made it very easy to trace stages and direction of differentiation in various tissues in hand sections.

## MORPHOLOGY

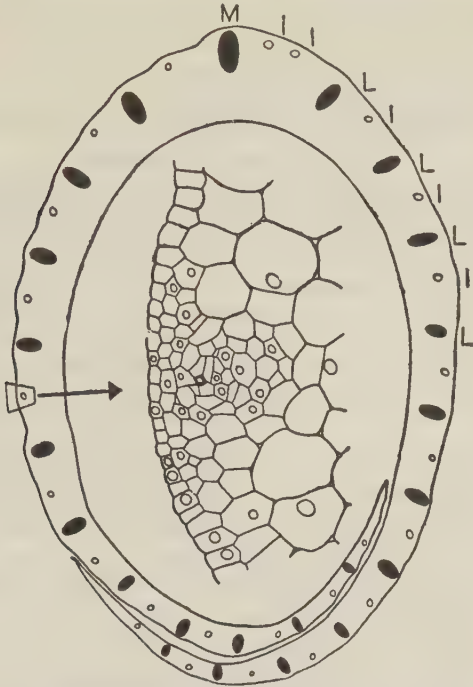
For the examination of the apical region a plant of about the maturity shown in Text-fig. 1 was chosen, where about the 10th leaf (counting the coleoptile as the first) has been exposed as far as the ligule. In more mature plants the new



TEXT-FIG. 1. Plant at about the same age as those which were used for the examination of shoot development. The plant shown has the 8th leaf from the coleoptile exposed as far as the ligule. A, latest leaf with lamina fully exposed; B, 7th leaf, lamina and sheath fully elongated but internode below quite short; C, 6th leaf, lamina and sheath fully elongated and internode has commenced extension; D, 5th leaf, beginning to die at the tip—internode fully elongated and firm with roots well out at base. X, position of apex. (Approx. nat. size.)

primordia are liable to be affected by pressure from surrounding leaves, and in rapidly growing material the growing point may be developing as an inflorescence.

In the adult leaf the lamina is usually widest about three-quarters of the distance from the tip to the ligule, and in a very young leaf the tip is rather more solid than the rest. In the lamina the large lateral veins alternate with a number of smaller intermediate ones. There is a progressive union of veins



TEXT-FIG. 2. T.S. of a leaf sheath, not quite mature, to show how the small intermediate veins, I, alternate with the larger basifugal lateral ones, L. Inset, one of the 'intermediates' to show detail. ( $\times 45$  and  $250$  approx. respectively.)

towards the tip and the base, and in the region of the ligule the intermediates unite with one another, so that in the sheath there is usually only one between each pair of laterals (Text-fig. 2). The sheath unites with the axis over a wide insertion, later leaves wrapping as much as one and a half times round the axis; alternate leaves overwrap in opposite directions.

The highest leaf (A in Text-fig. 1), which is fully exposed as far as the ligule, has a sheath which is still growing and is soft and pliable, especially at the base. A very short internode separates this leaf from the next below. The sheath of the next lower leaf, B, is fully elongated and firm, while the internode below its insertion is commencing to elongate rapidly: the top may already be quite firm, but the base extremely soft. Both the lamina and sheath of leaf C are fully elongated and firm, while the internode below has nearly reached its full length, and is firm and hard for the greater part of its length. The time taken for the internode to complete its development seems to increase with the maturity of the plant; thus in a small plant in which the

10th to 12th leaf from the coleoptile is just fully exposed as far as the ligule, the internode associated with the second leaf below is usually approaching its full length, whilst in a plant having the 16th to 18th leaf exposed, there may be a lapse of four or more plastochrones between the full expansion of the lamina and internode.

TABLE I

*Lamina, Sheath, and Internode Lengths for Maize Plants with the 8th Leaf fully exposed*

Average of four plants: measurements in cm.: leaves numbered from the coleoptile which is reckoned as the first leaf. 9th leaf has 50.0 cm. exposed.

Leaf

No.	Lamina.	Sheath.	Internode.	Notes.
3	15.8	7.6	—	Internode too short to measure.
4	25.2	8.5	0.3	Roots well out, internode firm.
5	33.5	11.5	0.5	Roots well out, internode firm.
6	41.5	16.4	0.7	Position of root initials indicated by brown dots on internode surface. Internode top firm, base softer.
7	56.1	20.1	1.1	Positions of root initials indicated by green-white dots. Internode soft.
8	66.6	19.4 (22)	0.4 (3)	Positions of root initials indicated by green pin points on internode surface. Internode very soft.
9	72.5 (77)	4.8 (23)	0.1 (6-10)	No indications of root initials.
10	51.8 (84)	0.8 (26)	— (10-15)	Internode too small to measure.
11	30.4 (90)	0.28 (28)	— (10-15)	Internode too small to measure.

The figures in brackets, which were obtained from plants which were allowed to develop further, indicate the dimensions the parts might be expected to reach when fully mature. The maximum mature length is reached by the 12-14th lamina and by about the 10th sheath and internode.

Table I gives the average measurements of four plants, in each of which the 8th leaf was just exposed as far as the ligule. It shows the way in which the elongation of the lamina precedes that of the sheath, which in its turn is followed by that of the internode below and then by the production of adventitious roots.

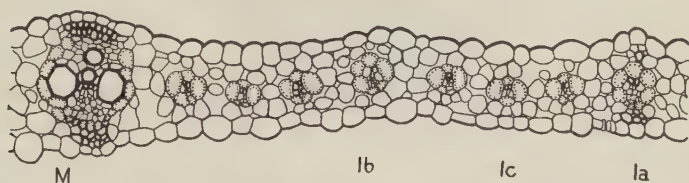
#### ADULT ANATOMY

*Leaf.* The maize leaf shows a typical grass leaf structure. Stomata occur on both sides, and the mesophyll is not differentiated into palisade and spongy layers. There is a mid-vein, but this is not a conspicuous feature of the adult leaf. In the lamina and sheath may be recognized a number of relatively strong lateral longitudinal veins (referred to as laterals or basifugals), whilst between the median and the first lateral bundles, and again between



each pair of laterals, there are a number of smaller veins, the intermediates (or basipetals). These have a well-defined sheath and usually contain one or more tracheids (with no metaxylem flanking vessels) and a phloem element or two. Text-figs. 2 and 3 show the distribution of the median (M), laterals (L), and intermediates (I) in the sheath and lamina respectively.

*Internode.* In a mature internode there is a general groundwork of parenchyma, each cell of which is a more or less flattened cylinder. In the sub-peripheral region is a band of more lignified cells forming the sclerenchyma.



TEXT-FIG. 3. T.S. of a small portion of a more distal part of the lamina. M, mid vein; Ia, Ib, Ic, three types of intermediate veins. ( $\times 140$  approx.)

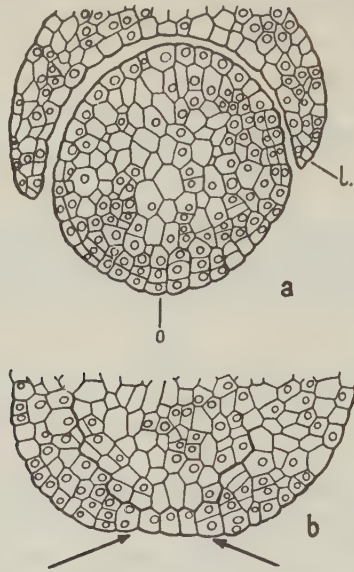
Under the lignified epidermis will be found small patches of sclerenchyma, alternating with patches of chlorenchyma, of a depth of some 4–5 cells. The centre of the internode is occupied by median trace bundles going to leaves above, whilst towards the outside are smaller and more numerous bundles, some of which are continuations of the intermediates of the leaf immediately above. In a transverse section it is impossible to distinguish with certainty between the smaller lateral and intermediate bundles, although those having no protoxylem should be considered as being probably in continuity with the intermediates of the leaf above.

At the base of the internode, just *above* the insertion of a leaf, will be found a network of anastomosing bundles, linking up the vertically running bundles, apparently indiscriminately. This is shown in Pl. VII, Fig. 1, where the network has been exposed by 'retting' the axis and washing away the decayed parenchyma (Evans, A. T., 1928, modified). Pl. VII, Fig. 2, shows the base of a mature plant split in half and treated in the same way. The anastomosis occurs just above the union of a leaf with the stem, and does not involve the bundles of this leaf. The roots are related to this anastomosis rather than to the leaf below.

#### *Developmental anatomy of the leaves and axis.*

*Apical development.* The apex of maize is a relatively pointed structure, with the primordia arising a little way back from the extreme tip (Pl. VII, Fig. 3). In the photograph the cells at A are a little larger than the rest and stain comparatively lightly, suggesting that even as far forward as this there is some indication of the beginning of vacuolation of the interior. The outer

layer, B, just under the 'dermatogen',<sup>1</sup> is composed of cells with rather darker-staining contents, suggesting that they are more meristematic<sup>1</sup> than the inner cells. The first sign of a new primordium is the appearance of periclinal



TEXT-FIG. 4 (*a* and *b*). T.S. apex showing new primordium arising by dermatogen and sub-epidermal divisions. *l*, edges of 1st primordium. (*b*) T.S. apex at slightly lower level than (*a*) through the insertion of primordium *l*. Arrows indicate the position of the leaf edge where primordium *l* is spreading round the axis by divisions in the dermatogen and sub-epidermal cells. ( $\times 280$  approx.)



TEXT-FIG. 5. Diagram illustrating the way in which the insertion of the primordium changes with age.

divisions in the slightly elongated cells of the dermatogen at one point of the apex (Pl. VII, Fig. 3, *c*). This is immediately associated with divisions in the cells constituting the sub-epidermal tissues B in the photograph (see also Text-fig. 4). The small emergence so formed is quickly converted into a

<sup>1</sup> Throughout this paper the term 'dermatogen' is used for the outermost layer of cells, although this layer undergoes periclinal divisions; and the phrase 'a meristematic cell' is used to mean one which (*a*) is actively dividing, (*b*) has a relatively unvacuolated protoplast, the vacuoles appearing as small grains, threads or perhaps as very small spherules, and (*c*) possesses a large nucleus-cell volume ratio.

collar surrounding the axis by the rapid lateral spread of similar divisions in the dermatogen and sub-epidermal cells to either side (Text-fig. 4*b*). As the collar spreads laterally, the first formed part continues growing at the tip and the new primordium begins to fold over the apex as a small hood.

The continued development of the tissue at the primordium tip can be seen in the photomicrograph Pl. VII, Fig. 3 at E, where the cells are smaller, more deeply-staining, and obviously more meristematic than the basal ones and those of the axis at the insertion.

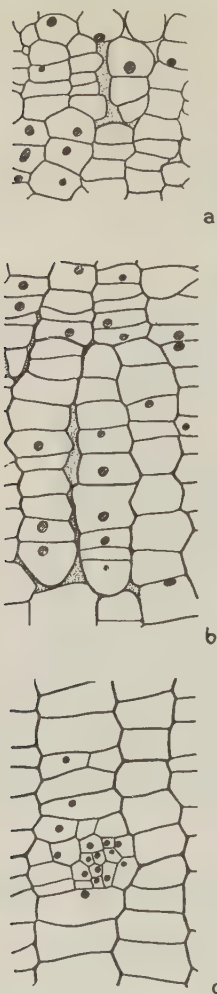
*Further growth of the primordium and axis.* Two marginal meristems run down the entire length of the primordium. It is the production of this new tissue (some obviously arising from the dermatogen at the edge) which enables the young leaf to keep pace with the rapidly widening axis. It is the marginal increase of the leaf which later (from about the time the leaf is the 3rd or 4th from the apex) helps to cause the lamina-edges to overlap: it is also partly the cause of the slightly ovate shape of the adult leaf.

In the axis the first obvious change is an increase in diameter. At the insertion of the 3rd primordium it is nearly two and a half times the diameter it was when the primordium was first visible as a distinct upgrowth. The increase seems to be mainly due to divisions in the cells towards the periphery, although a few divisions in the centre, coupled with the gradual increase in size of the cells in this region, also contribute. At the same time the cells in the base of the primordium are dividing, bringing about a change in the insertion of the young leaf, which instead of being a lateral outgrowth, projecting almost at right angles from the side of the axis, becomes a concentric structure appearing at one stage to arise vertically from a ledge on the axis (Text-fig. 5).

The continued activity of the outer tissues of the axis is seen in transverse sections, the more peripheral cells appearing smaller, more angular, and rather more deeply staining than the inner ones.

The main growth of the axis is at first chiefly in the transverse direction, although there is always some longitudinal increase. In the 'disc of insertion' of the 4th leaf from the apex, the cells of the axis begin to divide more and more frequently in horizontal planes, giving small vertical files of two or three cells. This first occurs in the centre of the axis, but soon spreads towards the periphery. Later, in a position towards the lower half of the disc of insertion of the primordium, there commences a more vigorous formation of files of cells by the acceleration of the cell divisions in this position (Text-fig. 6). This is merely a more vigorous and more localized expression of what has been previously taking place generally throughout the axis. It is this production of cell files (a long-continued process) which results in the formation of the internodes. Thus, in maize, the internode really corresponds to the lower half of the disc of insertion of the leaf (Text-fig. 14). At first the effect of the increase in axis length merely appears as an increasing depth of insertion of the primordium, but by the time the young leaf has become the





TEXT-FIG. 6 (a-c). Formation of files of cells leading to the production of internodes. (a) R.L.S. of the whole vertical depth of tissue between the 7th and 8th leaves from the apex. (b) R.L.S. portion of the tissue between the 8th and 9th leaves. (c) R.L.S. portion of the tissue at the base of the 12th internode, showing the production of an anastomosing nodal bundle in one of the vertical files of parenchyma. ( $\times 200$  approx.)

5th from the apex, the beginning of a definite internode may be distinguished. It is as though there were an expansion of the whole of the lower half of the primordium and the enclosed axis.

In the axis it is the formation of vertical files of cells which is the main factor in determining the vertical course taken by the provascular strands, especially those originating early. In the nodes, where the parenchyma does not form long files, the strands maintain their original courses or become more horizontal by the transverse expansion of this region (Text-fig. 11).

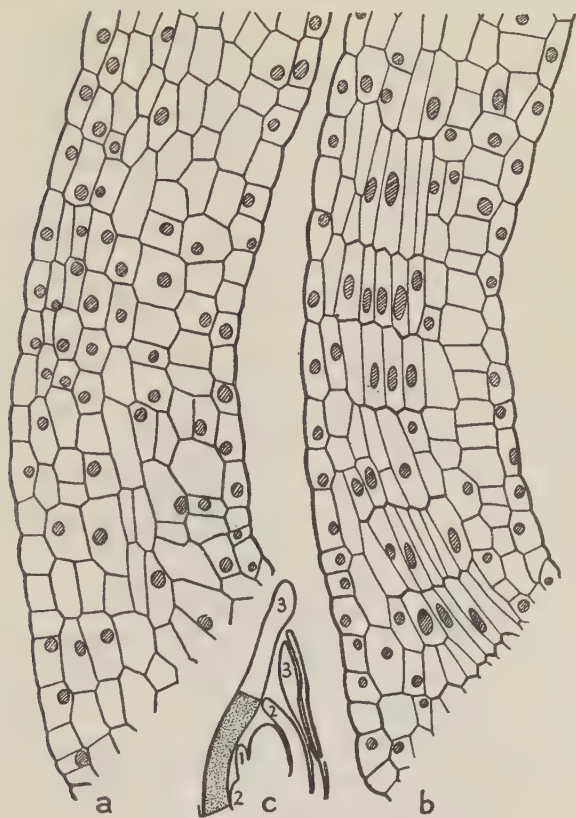
The transverse divisions result in an axial tissue composed of long vertical files of cells, which are not meristematic in the same sense as are the cells at the stem apex or the tip of the primordium. They are larger, with larger vacuoles and more conspicuous walls. They have a much smaller ratio of nucleus to cell volume. As will be seen later, although they appear to be differentiating into parenchyma, they are capable of becoming more meristematic again and giving rise to provascular strands. They appear to be passing through an intermediate phase and are so little differentiated that, given appropriate conditions (food, aeration, &c.), they can return to the meristematic condition, but once the process of differentiation has passed a certain stage, they cannot readily become meristematic again.

In the primordium whilst the cells of the marginal meristems continue to divide in all planes, those towards the centre soon begin to divide more and more exclusively in a transverse plane, resulting in long files of cells (Text-fig. 7). The so-called intercalary 'meristem', which later becomes confined to



the base of the leaf (together with the axis), is merely a wide zone of these actively dividing, partly vacuolated cells.

The marginal meristem itself is also progressively transformed into an active tissue of partially parenchymatized cells, dividing mainly at right



TEXT-FIG. 7 (*a*, *b*, and *c*). L.S. of the base of the 3rd primordium. (*a*) taken slightly off the median plane, to show that the cells constituting the primordium are already in longitudinal rows. (*b*), median section showing the initiation of the median provascular strand from the axis into the primordium. Note that the cells of the strand at any level seem to be derived from one cell. (*c*), low-power diagram of the apex concerned to show position of tissue drawn in (*a*) and (*b*). (*a* and *b*  $\times 260$  approx., *c*  $\times 32$  approx.)

angles to the long axis of the leaf to give long cell chains. By about the time the primordium is the 5th from the apex, no truly meristematic tissue is left.

**Bud formation.** Long before an axillary bud can be seen as an emergence, there is a production of radially running files of cells in the axis. These files of cells can be seen in transverse sections at the base of the 2nd primordium (Pl. VII, Fig. 4), although (in this series of sections) the bud only appears as a raised structure in the axil of the 4th leaf. In the axil of the 5th primordium the bud is much more plainly evident, although it is still without primordia.

These radial files (Text-fig. 8) soon become small-celled and deeply

staining towards the periphery and are situated at the base of the disc of insertion of the leaf above, seemingly in continuity with and beneath the marginal meristems of this leaf. Thus the axillary bud seems to be associated with the leaf above and its continued marginal growth, and not with the leaf in



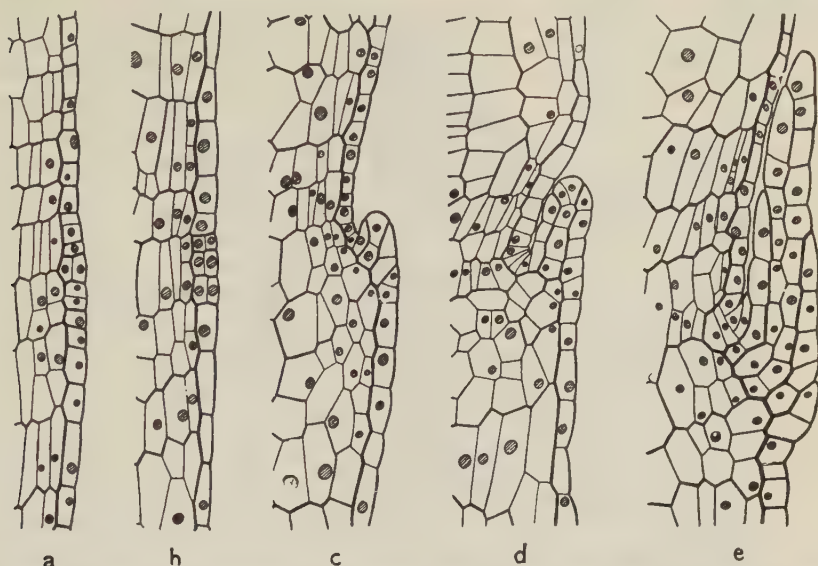
TEXT-FIG. 8. L.S. 4th primordium from a vigorously growing apex, showing the development of the bud at the base of the insertion of the margins of the leaf above. ( $\times 410$  approx.)

whose axil it appears.<sup>1</sup> Even when the leaf is separated from the leaf above by an internode, this separation is merely the result of the expansion of the lower half of the disc of insertion of the leaf above. The final development of the bud represents the last activity of a phase of growth which started on one side of the apex at the point where the leaf was initiated and passed down the leaf-internode unit and then reached its conclusion in the formation of a bud on the other side of the stem. When the provascular strands differentiate from the bud into the axis, they differentiate in the base of the internode and not in

<sup>1</sup> Compare Skutch (1927), who says that the buds of *Musa sapientum* are not found in the axils of the leaves but at the union of the margins of the laminae at their insertion.

tissue associated with the insertion of the leaf below, in the axil of which the bud is found.

*Development of the ligule.* Towards the end of the 4th or the beginning of the 5th plastochrone epidermal cells in the position to be occupied by the



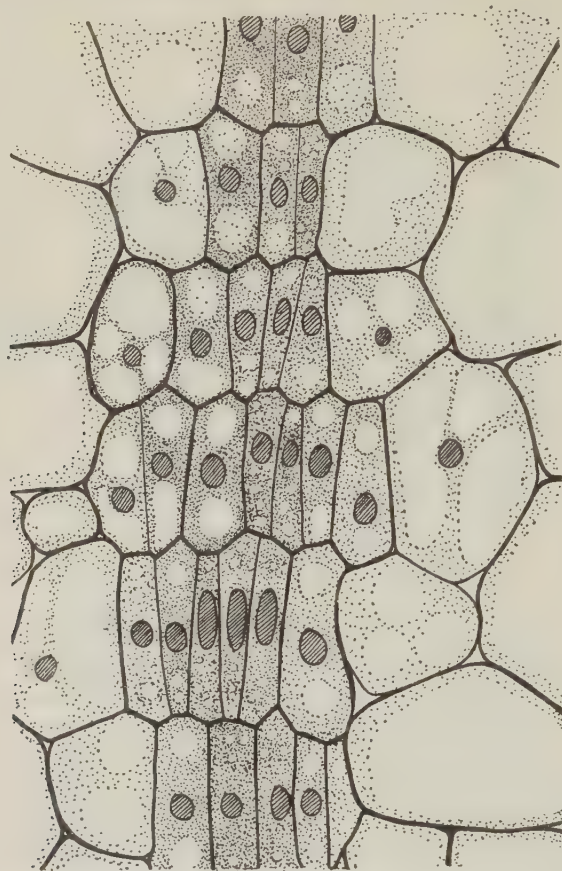
TEXT-FIG. 9 (a-e). Development of the ligule from the epidermal layer. ( $\times 290$  approx.)

future ligule become more densely staining and more meristematic. Later they divide by periclinal divisions (Text-fig. 9, a and b), forming a small up-fold; further divisions of these cells produce the structure seen in Text-fig. 9, d and e. Although divisions in the sub-epidermal cells accompany the periclinal divisions in the epidermal cells, the ligule itself is derived solely from the epidermis. This is shown in longitudinal sections, partly by the continuity of the sub-epidermal cells across under the ligule, but also by a similar continuity of the rather thicker original inner wall of the epidermal cells (Text-fig. 9).

*Development of the main and lateral provascular strands in the leaf primordium and internode below.* In serial transverse sections of the apex it is usually in the 2nd primordium that one first sees a median provascular strand. The strand seems to result from divisions of a single chain of cells as most of the cells forming the strand have coincident end-walls (Text-fig. 10). Later the strand is increased mainly by division of the more central cells in both tangential and radial longitudinal directions, so that even if not everywhere originally derived from one cell chain, the strand now has a stratified appearance when seen in sections cut in longitudinal planes.

The median strand going to the primordium develops basifugally, at least in the upper part of its course, and is produced directly out of the slightly

vacuolated cells of the axis and primordium. Repeated divisions occur, during which the nuclei appear denser and the cytoplasm increases and becomes more deeply staining. The strand for the next primordium to be produced (primordium 'o') can be seen when the new leaf is represented by



TEXT-FIG. 10. Initiation of the median provascular strand from the partially parenchymatized cells of the axis. ( $\times 500$  approx.)

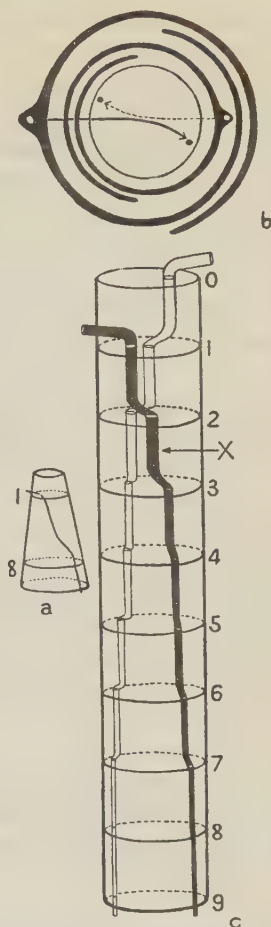
only a few dermatogen divisions. In serial transverse sections the highest level at which this strand can be seen is between the insertion of the 2nd and 3rd primordia, when it appears as a group of 4-6 cells in which the outer ones tend to be slightly larger. Tracing such a strand down the axis, the number of cells in it increases considerably, until, when the level of the insertion of the 6th primordium is reached, it contains about 20 cells, as well as 3 or 4 well-defined phloem elements which are easily recognized in sections stained in safranin and fast green by their deep-green colour. The strand usually unites somewhere at this level with one of the more centrally placed laterals from a



leaf inserted just above this level. Longitudinal sections cut in the plane of the median strands often show the provascular strand well defined and leading up towards the new primordium, but becoming progressively less differentiated until, in the region immediately below the insertion of the primordium, it cannot be distinguished from the surrounding cells, except that just beyond the end of the strand the cells have rather darker staining nuclei.

By the time the primordium is appearing as a distinct swelling on the side of the apex (Text-fig. 5, *b*, and Pl. VII, Fig. 3), i.e. late in the plastochrone, the provascular strand is extending into its central tissue. From longitudinal sections it can be seen that in the leaf the strand is formed by divisions of cells which at first looked as if they were going to become ordinary parenchyma (Text-fig. 7, *b*). However, here the stratified appearance is less marked than in the axis owing to the more rapid pulling out of the strand and to the less vacuolated appearance of the cells amongst which they lie and from which they are derived.

If the median strand is followed downwards from the leaf insertion, it swings sharply in towards the centre of the stem, where it follows a vertical course for some distance before moving towards the side opposite to that from which it entered. It does not go to a position diametrically opposite the point of entry but a little to one side, so that the median strands from leaves on one side pass those from the leaves on the other, as illustrated in Text-fig. 11. When it has reached the peripheral region, it runs down amongst the *laterals* of the leaves inserted opposite and below that from which it is derived. The strand finally unites with one of these laterals, usually the next or next but one to the median. Thus the median vein of each leaf appears to be



TEXT-FIG. 11 (*a-c*). Diagrams illustrating the course of the median bundle in the axis before and after the production and extension of the internodes. (*a*) shows a single bundle before extension, and (*b*) and (*c*) show how the median bundles pass to the opposite side of the axis as they are traced downwards from their point of entry in the adult axis. X, where the bundle is in the centre of the axis, is the level used for the views shown in Text-fig. 16.

linked lower in the axis with a lateral bundle of an older leaf inserted on the opposite side of the stem.

It was only possible to trace the median bundles from the primordium to a point of union in one or two series of sections cut from plants which were still little more than seedlings. Determinations of this nature in adult plants with longer internodes require very long series of sections. There is no definite plan underlying the linkages of the medians with the laterals. Sometimes linkages occur early: Text-figs. 17 and 20 show the median of the 4th leaf from the apex (Text-fig. 19) uniting with the 2nd lateral of the 5th leaf from the apex (Text-fig. 18), the union taking place at a level just below the insertion of the 7th leaf on the axis (Text-fig. 20). More usually the two linked leaves are farther apart and the union takes place at a lower level. In the same plant the strand going to primordium 'o' (numbered upwards, the 9th, inclusive from the coleoptile) was traced down the axis to the insertion of the 7th leaf from the apex without there being any union: the strand could not be traced below this level.

In every case where it was possible to trace the union it was always with a lateral bundle. In more mature plants there appeared to be a greater number of leaves separating the primordium and the leaf with the lateral of which it was linked. Furthermore, in the case of these higher leaves the lateral as well as the median went down through a greater number of internodes before the two united. In cases where it was impossible to trace the median strand to a point of union with another strand, it could be followed 8 or more internodes from its point of entry, to a position where it was amongst the laterals and presumably would soon unite with one of them. In such a region, however, the basipetal bundles are forming and the provascular ring (see p. 261) is being initiated at the internode base, so that there is also the possibility of the bundle uniting with either a basipetal bundle or the provascular ring in this region.

Thus it seems that the lower part of the course of the median strand is liable to considerable variation. All that can be said is that the median entering the axis takes a downward course and generally unites with a fairly centrally placed lateral derived from a leaf situated below and on the opposite side, the union taking place some 2 to 8 or more internodes below the insertion of the primordium.

*The lateral strands.* The lateral strands are also produced by repeated longitudinal divisions of partially parenchymatized cells. They differ from the median strands, however, in that they are not initiated low down in the axis. They appear to originate either in the disc of insertion of the leaf, as reported by Bugnon (1924) for *Dactylis* (and by Guichard (1929) for *Carex*), or in the leaf base just above its insertion. Very careful examination even of longitudinal sections failed to elucidate this point. Although there remains this doubt as to the exact position of their first appearance, their development up towards the tip of the leaf and downwards in the axis is easier to see. The order of

appearance of the laterals is from the proximity of the median, outwards, so that the earliest lateral strands are those on each side of the median. When transverse sections of the apex are examined it will be seen that the edges of the lamina are still meristematic and without vascular strands, and as this meristem gives way to partially parenchymatized tissue, progressively from the centre towards the edges, new laterals arise in it in the same manner as those which occupy the more central tissue.

After they have been differentiated in the base of the primordium or in its disc of insertion, they spread up into the primordium and downwards in the axis. They do not approach the centre of the stem, but maintain a vertical course: there is little to suggest the much quoted 'palm' type of distribution seen in many monocotyledon axes and which Haga (1922) apparently suggests as being applicable to maize.

Those bundles next to the median are set deepest in the axis, whilst those derived from nearer the edges of the leaf are developed each a little more towards the periphery, as indicated in Text-fig. 12.

*Development of the basipetal or intermediate strands.* Anastomosing 'strands' at the nodes. The basipetal or intermediate strands are found first at the tip of the leaf, later down the lamina, then in the sheath, and finally in the internode. At the time of their appearance at the leaf-tip, the tissues there are still comparatively young, having been lately derived from the tip meristem. It is at the tip of leaf 4 that the first indications of the production of basipetals are seen; very late in the plastochrone signs may be seen in leaf 3. They are being initiated at a time when the lateral strands on each side of them have just penetrated through to the leaf-tip and the first protophloem has just differentiated in these lateral strands in this region (Text-fig. 13). The basipetal strands do not terminate blindly at their distal ends, but each is always connected with another strand which may be either the median, a lateral, or another basipetal. Transverse anastomosing strands between all these types of strands originate in basipetal succession immediately behind the initiation of the basipetals.

More than one basipetal strand is produced between each lateral in the wider part of the lamina, where the partially vacuolated cells of the longitudinal files have divided longitudinally and perpendicularly to the plane of the leaf so as to cause the original laterals to be separated by a considerable width of undifferentiated tissue. Thus in a very large leaf, e.g. one between the

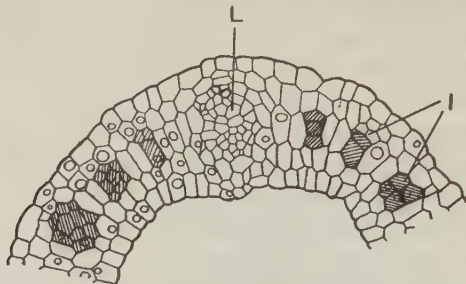


TEXT-FIG. 12. Diagrammatic T.S. of the adult axis just below a leaf insertion to show how the median bundle is set deep in the axis, while each successively younger lateral bundle is nearer the periphery, with the exception of those derived from the overlapping margins of the leaf.



10th and 15th from the coleoptile, there may be 12 or more intermediates between each lateral.

The first basipetals originate in the comparatively meristematic tissue at the leaf-tip and later develop through more vacuolated pseudo-parenchymatous tissue as they differentiate down the leaf. Those nearest the median are the



TEXT-FIG. 13. T.S. portion of the edge of the distal part of the 4th leaf from the apex, to show the initiation of the basipetal intermediate strands when the first protophloem has reached the tip in the lateral bundles. L, lateral bundle, the protophloem shown with thick walls. I, basipetal intermediate strands developing from partially parenchymatized ground tissue. The thickening of the cell walls and the shading of the contents are for emphasis only; except for their size and shape, the cells are practically indistinguishable from the surrounding cells at this stage. ( $\times 380$  approx.)

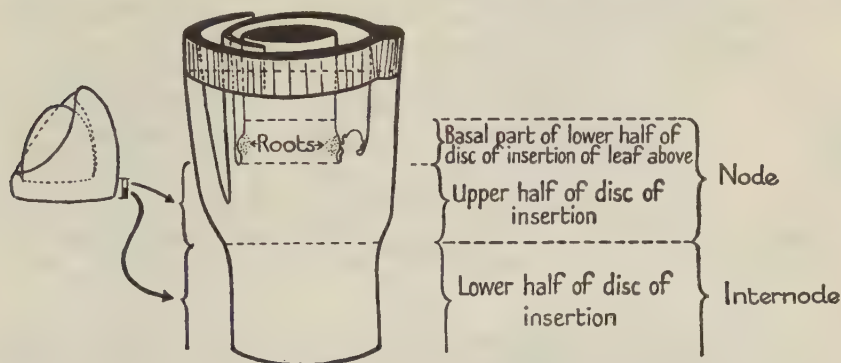
first to form, and at any given time they have penetrated the lamina to a greater distance than those nearer the margins. At the margins of the lamina the commencement of initiation of intermediate strands would naturally be later since it appears to wait upon the upward differentiation of the first protophloem in the adjacent laterals, which themselves are later to develop in this region.

The intermediate strands are reduced by progressive union towards the base of the lamina and again in the ligular region, until in the sheath only one lies between any pair of lateral strands. An exception to this may be found in the sheath of very large leaves (10th–15th from the coleoptile), where sometimes 2 or even 3 intermediates occur between the median and the adjacent lateral strands. The basipetal strands enter the internode when the leaf is about the 5th from the apex, and continue their development down the still relatively meristematic sub-peripheral region.

When the intermediate strands reach the base of the internode, they take a course towards the centre of the axis and finally differentiate towards one or more laterals (or a median). Basipetal strands coming from a position nearer the median in the leaf reach the base of the internode first, being found there about the time that the leaf is the 7th from the apex, or 6th if the plant has been fixed late in the plastochrone. By the time the leaf is the 8th from the apex the tissue at the base of the internode is permeated by an extremely complex network of anastomosing strands, developed from the lower cells of the files forming the internode (Text-fig. 6, c).



The fact that the basipetals differentiate out of the base of the internodal tissue and the roots emerge from here creates a tendency to associate this region with the node below, but in reality such a nodal region consists of the lower part of the internode (itself derived from the lower half of the disc of



TEXT-FIG. 14. Diagram illustrating the relationship between the newly developed primordium with its disc of insertion and the adult 'node' and 'internode'.

insertion of the leaf above) and the upper half of the disc of insertion of the leaf below, as shown diagrammatically in Text-fig. 14.

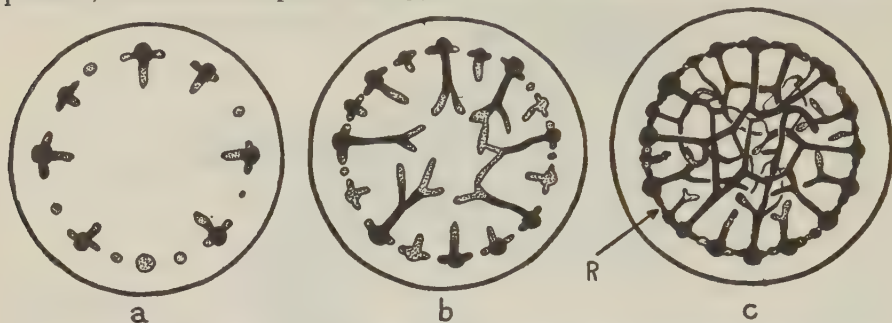
It would appear that, in the upper part of the disc of insertion of the leaf below, the young parenchyma is differentiated too far to become meristematic again, so that even the first basipetals of the leaf above cannot differentiate across this zone, and are thus prevented from penetrating this internode.

When the first basipetals reach the base of the internode and continue to form horizontally towards the centre, they show a slight tendency also to spread out sideways in the sub-peripheral tissue in which they are developing. As more and more strands come down, the lateral spread of one links up on each side with that of neighbouring strands, as indicated in Text-fig. 15. Thus examination of the base of the 8th internode shows an almost continuous ring of meristematic tissue enclosing the main and laterally derived bundles (Text-fig. 15, c). It is here that the root initials have their origin, being initiated in intimate contact with the meristematic ring and in tissue derived from the lower half of the disc of insertion of the leaf above, with which they are clearly to be associated.

#### *Differentiation of vascular bundles from provascular strands.*

Study of the development of the vascular strands is complicated by the fact that the bundles sometimes have a different appearance in different parts of their course, especially with regard to the associated sclerenchyma. Furthermore, any particular bundle is not in the same stage of development throughout its course. To avoid needless repetition, the development of the main leaf trace bundle is described first, and later any points of difference between this type and the others are indicated. The description applies to the bundle at a

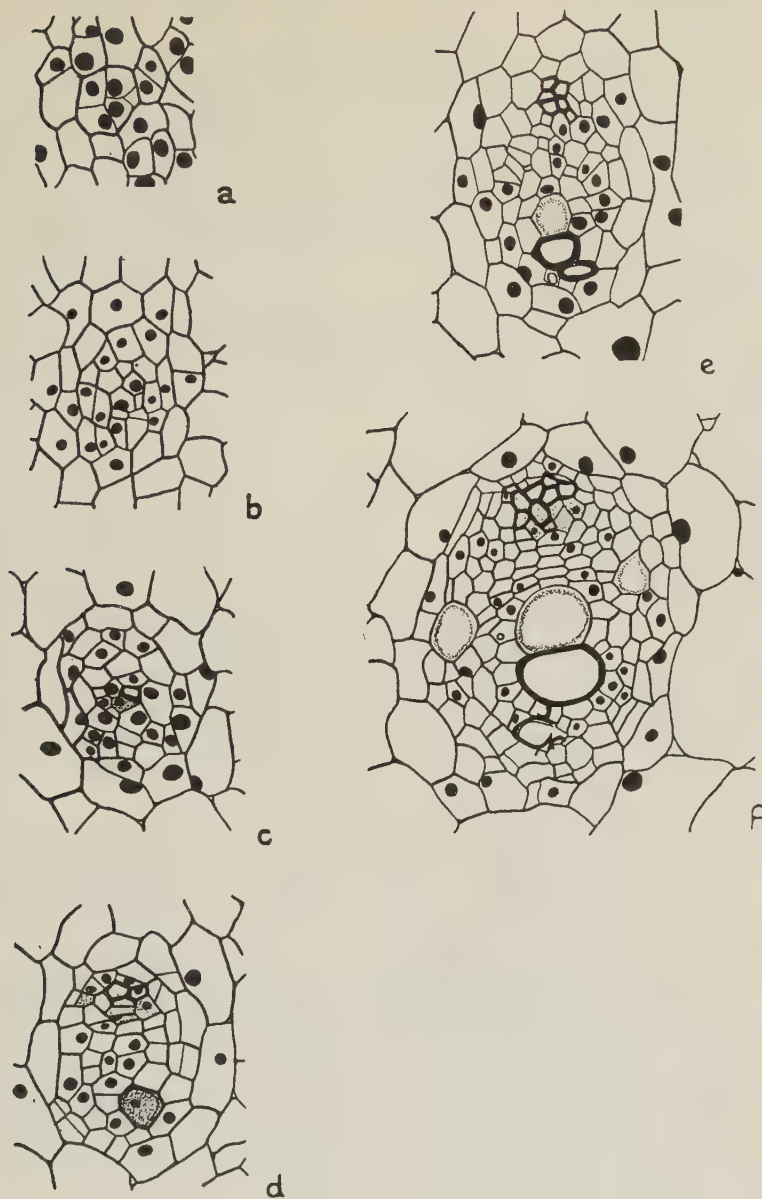
position where it is fairly well in the centre of the axis, i.e. about half-way down the second internode below the insertion of the leaf into which it leads (x in Text-fig. 11, c). Of course in very young stages this 'internode' is not present; then the description will apply to the bundles at the lower half of the



TEXT-FIG. 15 (a-c). Diagram illustrating the formation of the nodal anastomosis and ring of meristematic tissue by the basipetal intermediate strands when they reach the base of the internode. R, meristematic ring. The lighter-shaded parts indicate the regions where the strands have only lately been initiated.

disc of insertion of the appropriate leaf, at the position where the future internode will develop. Since all the observations (and the figures) refer to equivalent levels, this gives the nearest approach possible to direct observation of development of the same bundle.

The first signs of differentiation of any elements in the bundle occur on the outer side (Text-fig. 16, c). One or two cells have contents which stain exceedingly darkly with fast green, but later are mostly lost; their walls are now slightly thickened and more deeply stained by fast green. These first protophloem elements increase in number until about 5 are seen in transverse section. In longitudinal section they are elongated structures with numerous thin areas on their longitudinal walls. The end walls of each phloem element coincide with those of neighbouring elements as well as with the ends of the still undifferentiated cells of the strand. (This can be seen very easily if thick (10–12  $\mu$ ) microtome sections are taken through Eau de Javelle before staining, then by focusing up and down, a number of phloem tubes may be examined.) At this stage the first protoxylem is seen developing on the inner side of the strand; Text-fig. 16, d, shows the xylem differentiating in the bundle running to the 3rd leaf from the apex. More elements are produced until about 12–15 protophloem and 4–5 protoxylem elements may be found. As the formation of vertical files of cells is commencing in the ground parenchyma, the bundles are being extended, leading to the collapse of the first-formed protoxylem, so that it is impossible to be sure of the exact number which have so far been formed. By now the bundle will appear larger in transverse section owing to the cambial-like division of the cells between the protophloem and protoxylem. In Text-fig. 16, d, e, and f, the resulting radial arrangement of the cells in this region can be seen.



TEXT-FIG. 16 (a-f). Transverse sections to show differentiation, in plastochrone intervals, of the median strand to successive leaves taken at equivalent positions in their courses (position x in Text-fig. 11). (a), strand developing basifugally to primordium 'o' which as yet is only represented by a few dermatogen and sub-epidermal divisions on the side of the apex. (b) to (f) show the strands running to the 1st to 5th primordia respectively. ( $\times 533$  approx.)

In Text-fig. 16, *f*, is shown the commencement of the development of the two large flanking metaxylem vessels. Soon after this many of the phloem-mother cells divide, giving files of sieve tubes alternating with companion cells. The cells of the central tissue near the protoxylem vessels differentiate into xylem tracheids, whilst any undifferentiated cells remain as parenchyma separating the last metaphloem from the metaxylem.

Early in its development the bundle is enclosed in a sheath of rather larger cells (Text-fig. 16, *a-e*). Their later development and lignification gives the characteristic sclerenchyma sheath.

During the development of the median bundle in the leaf the same sequence of events occurs, but with a rather smaller production of protophloem. There is also a tendency for the ensheathing cells to be more prominent: they do not divide, or at least not conspicuously, nor do they lignify to give a 'sclerenchymatous' sheath.

#### *Course of differentiation of the median bundle.*

*Protophloem.* Differentiation of the median bundle from the provascular strand does not commence simultaneously throughout its length. Protophloem is first found in the region where the bundle unites with the lateral of a leaf below. For example, in one plant fixed towards the end of a plastochrone, in the 1st primordium there was a well-defined provascular strand without protophloem. At the level of the insertion of the 3rd primordium this strand had two well-defined protophloem elements, and four at the level of insertion of the 4th primordium. This basifugal differentiation of the protophloem is even more striking in the strand of primordium 'o', which is still represented as a few dermatogen divisions and the strand of which has not yet reached the base of the primordium. In this case the 1st protophloem was seen at the level of insertion of the 4th primordium from the apex, while at the union of the 6th leaf the same strand had three or four well-defined protophloem elements.

In that part of the axial course which lies below the internode associated with the leaf (the internode immediately below the leaf insertion) all the protophloem of the median strand seems to be developed basifugally, and by the time the leaf is fully exposed all the phloem has been formed in this region, no more being added when the leaf and its associated internode undergo their final maturation. Furthermore, this phloem looks different from that developed in the strand in the leaf and associated internode. Its irregular arrangement and its lack of conspicuous companion cells suggest that it is entirely composed of protophloem.

*Protoxylem.* Each protoxylem vessel is differentiated simultaneously throughout a considerable length of the bundle. The second vessel sometimes reaches nearer the tip of the primordium, possibly this is connected with the later derivation of the tissue at the extreme leaf apex from the short-lived tip meristem. After the first few protoxylem elements differentiation in



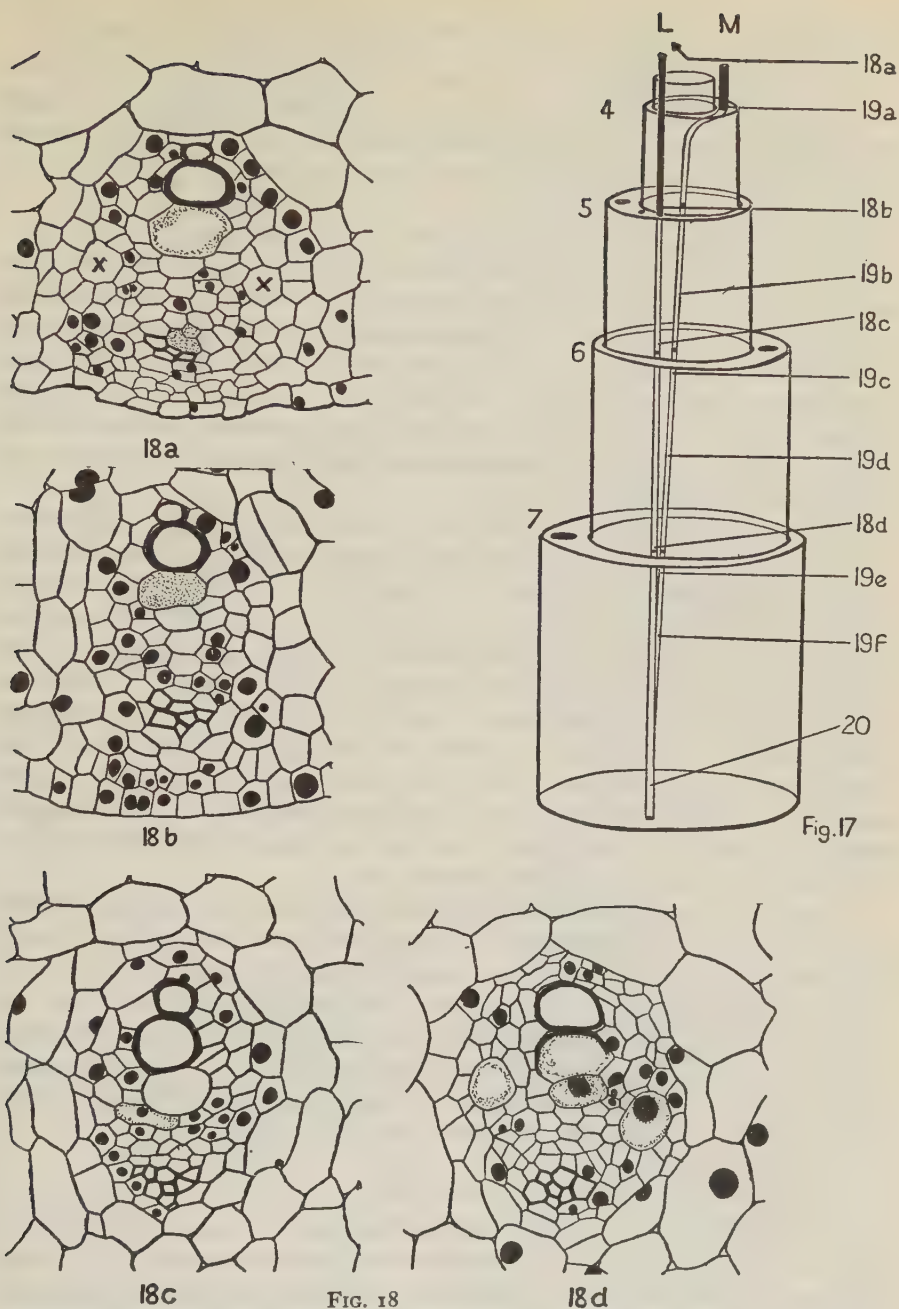


FIG. 18

TEXT-FIG. 17. Diagram to show how the 2nd lateral (L) of leaf 5 unites, in the case mentioned in the text, with the median (M) of leaf 4 at the base of the 7th internode. The numbers on the right show the levels which are illustrated in Text-figs. 18, 19, and 20.

TEXT-FIG. 18 (a-d). Transverse sections of the 2nd lateral of the 5th leaf, at levels shown in Text-fig. 17, to show (in conjunction with Text-fig. 20) the manner in which the strand develops basifugally in the lower part of its course. The presence of metaxylem flanking vessels (marked with crosses) in *a*, and their absence from *b* and *c*, foreshadow the later basipetal development of the bundle in the leaf-associated internode unit. ( $\times 533$  approx.)

the axis seems to be limited to that part of the course which lies below the internode associated with the leaf. In the leaf itself, however, at the time when the leaf is about the 6th from the apex, more protoxylem is formed, firstly at the tip and then progressively downwards into the internode below (the 'associated' internode).

*Metaphloem and metaxylem.* As the wave of cell expansion works down the leaf and associated internode, metaphloem and metaxylem develop basipetally from the still undifferentiated tissue in the strand. In the part of the course which is in the axis below the associated internode, the development of the two large metaxylem vessels and the small metaxylem tracheids seems to be initiated independently (Text-fig. 19, *d, e, and f*, taken with Text-fig. 20) so that it is quite possible to find in the same median strand proto- and metaxylem in the leaf, protoxylem alone in the associated internode, and proto- and metaxylem in the region below this.

The same general course of development is followed in the lateral bundles. In fact, in the internode, except for their lateness and their position, it would often be impossible to distinguish between the two types. In the leaf, at least, the protophloem is developed basifugally. Text-fig. 18, *d*, in conjunction with Text-fig. 20 shows the basifugal character of the development of the lateral strand in the lower part of its course at positions shown in Text-fig. 17. In Text-fig. 18, *a*, early stages in the differentiation of the metaxylem flanking vessels are shown. The fact that these do not occur at the levels at which Text-fig. 18, *b* and *c*, are taken, is a sign that the later development of the lateral strand in the leaf-associated internode unit is initiated high up and independently of events low in the course. The basipetal nature of this later development in this part is evident from Text-fig. 21.

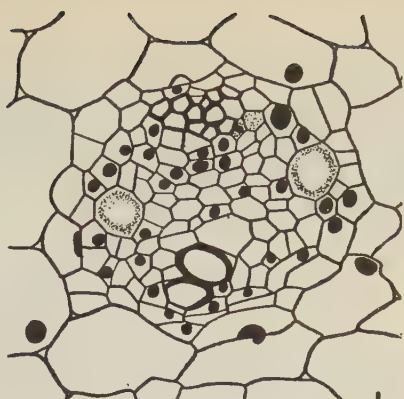
In the axis the intermediates are both initiated and differentiated basipetally and the final appearance of the earlier formed of them is almost exactly the same as that of the main and lateral bundles; in a section it is usually impossible to distinguish if any particular bundle is a basipetal or lateral. In the case of the later intermediates only metaxylem is developed, but others with two or three protoxylem elements may be found. In the nodal plexus all the xylem in these strands develops as metaxylem, since here they are late in forming and there is relatively little transverse expansion.

#### *Wave of final vacuolation and cell extension.*

At about the time the leaf then 6th from the apex is being exposed at its tip, there spreads down it a wave of vigorous elongation accompanied by the rapid maturation of the tissues. Commencing at the tip and passing down the lamina, this works down the sheath and internode, and is followed by the emergence of the ring of roots at the internode base. It is during this period that the stomata develop and also the chloroplasts and the large air spaces in the mesophyll. The same basipetal mode of development occurs in the bundles themselves. The final development of the median and lateral bundles



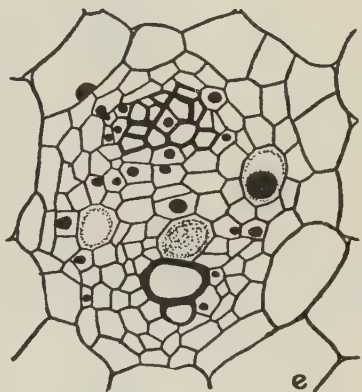
a



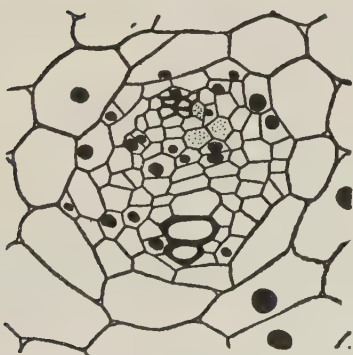
d



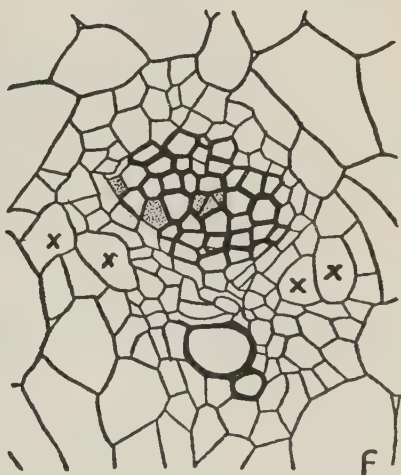
b



e



c



f

TEXT-FIG. 19 (a-f). Transverse sections of the median bundle of the 4th leaf from the apex taken at levels illustrated in Text-fig. 17 to show the basifugal development of the metaxylem and phloem (? all metaphloem) in the lower part of the course. The metaxylem here 19 (d, e, and f) is apparently initiated independently of the later initiation which occurs at the leaf-tip and travels down the leaf and upper part of the course in the axis. ( $\times$  533 approx.)

and the lignification of the sclerenchyma all take place basipetally. In the main and lateral bundles of the lamina, and perhaps the sheath and internode, even the last protoxylem develops basipetally (Text-fig. 21). In the case of the intermediate bundles all the tissues develop basipetally. In the lamina,

TABLE II. *Analysis of the State of Differentiation of the Tissues of the 6th Leaf from the Coleoptile*

Leaf 5 just fully exposed as far as the ligule; leaf 6 exposed 29 cm., total length at present 40 cm., plus sheath at present about 1 cm.

Distance (cm.) from leaf-tip	2	5	10	18	<i>a</i>		<i>b</i>		<i>c</i>
					25	30	34	37	
<i>Exposed to light</i> (29 cm. from tip) . . . . .									
<i>General tissues</i>									
Cuticle thicker than normal wall . . . . .						-----			
Stomata present . . . . .								-----	
Chlorophyll in mesophyll . . . . .							-----		
<i>Main and lateral bundles</i>									
Protophloem crushed . . . . .									
Secondary phloem 'adult' . . . . .									
Last protoxylem fully differentiated . . . . .									
Metaxylem flanking vessels lignified . . . . .									
Metaxylem tracheids lignified . . . . .						-----			
Sclerenchyma lignified . . . . .						-----			
<i>Intermediate bundles</i>									
Tracheids lignified . . . . .						-----			

The letters *a*, *b*, and *c* indicate respectively the position of the sections of the lateral bundles shown in Text-fig. 21, *a*, *b*, and *c*.

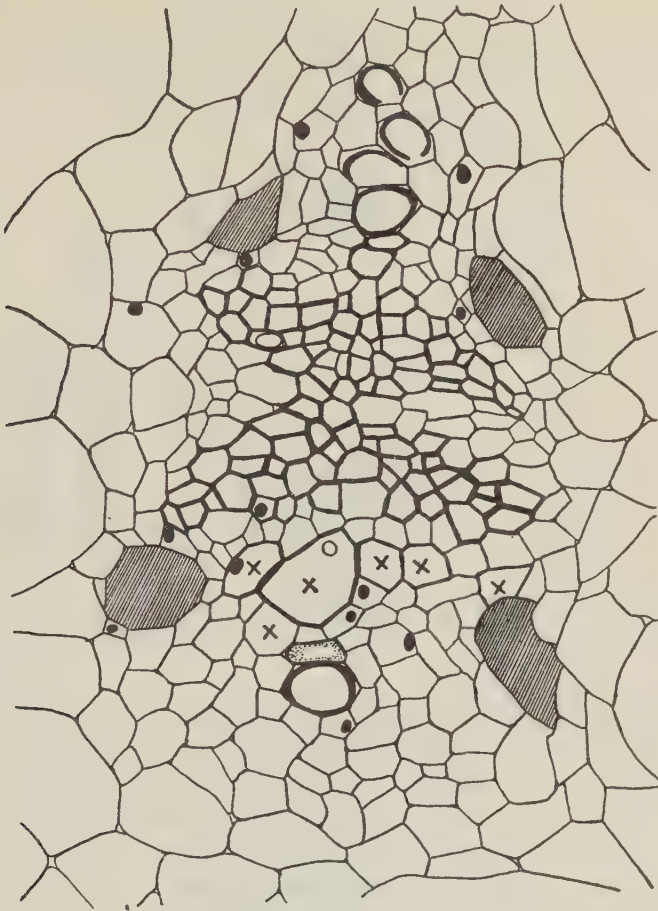
but not in the sheath and internode, all these changes seem to take place in the tissue when, or immediately before, it is exposed to the light as the leaf is growing up out of the centre of the surrounding leaves. Text-fig. 22 illustrates the course of this wave of last cell extension and maturation.

Table II gives an analysis of the state of differentiation of the tissues in a leaf which is exposed for 29 cm. of its present length of 40 cm. Text-fig. 21 shows transverse sections of a lateral bundle, the 2nd from the median, at levels 37, 34, and 25 cm. from the tip respectively. Text-fig. 18, *a*, in conjunction with 18, *b*, shows the first signs of the basipetal development of the metaxylem flanking vessels of a young leaf (still in the 5th plastochrone).

The progress of this wave of maturation down the bundles may create an impression that the top of a vessel may be fully mature while the lower end still contains protoplasts. In fact, however, throughout the internode, sheath, and lamina there are chains of overlapping vessels of varying lengths and at successive stages of development. The overlap accounts for the appearance sometimes seen in transverse sections, when a single vessel is replaced by two or even three. The actual length of overlap is often considerable, 1,000  $\mu$  being common in the lateral bundles of the sheath.



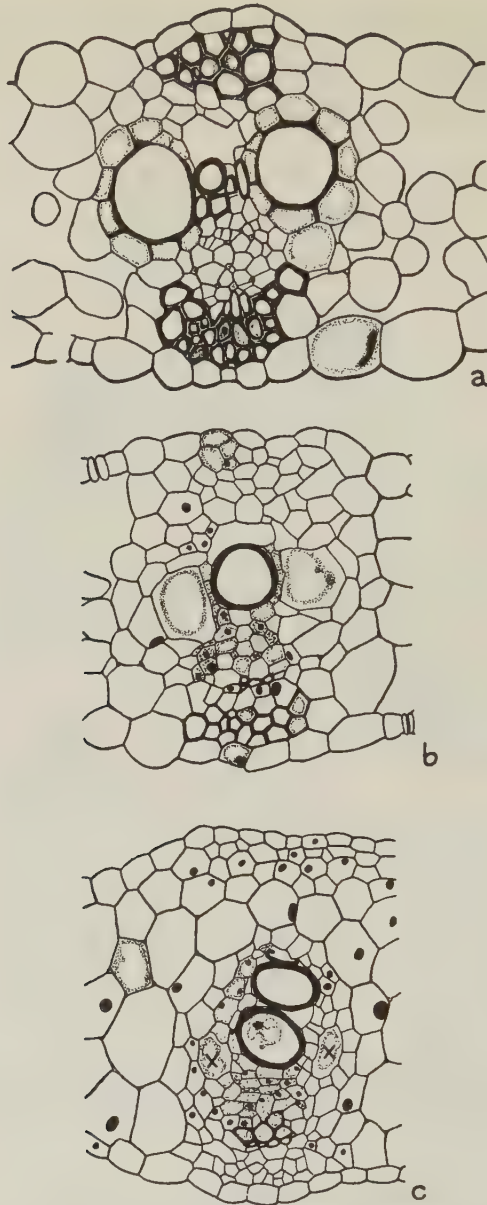
During the final extension of the internode the top appears firm and stiff while the base is limp and pliable. Hand sections placed in water or alcohol just coloured with safranin enable the basipetal progress to be followed in the



TEXT-FIG. 20. Amphivasal bundle formed by the union of the 2nd lateral (above) of the 5th leaf and the median (below) of the 4th leaf. The position, shown in Text-fig. 17, is at the base of the 7th internode. The metaxylem flanking vessels are shaded for emphasis only. ( $\times 533$  approx.)

maturation of the ground parenchyma, the development of chlorenchyma under the epidermis, the thickening and lignification of the schlerenchyma, the development of the cuticle, and the lignification of the epidermis. There is no doubt whatever that the maturation of the general ground tissues of the internode waits upon and is linked with the development of the leaf above.

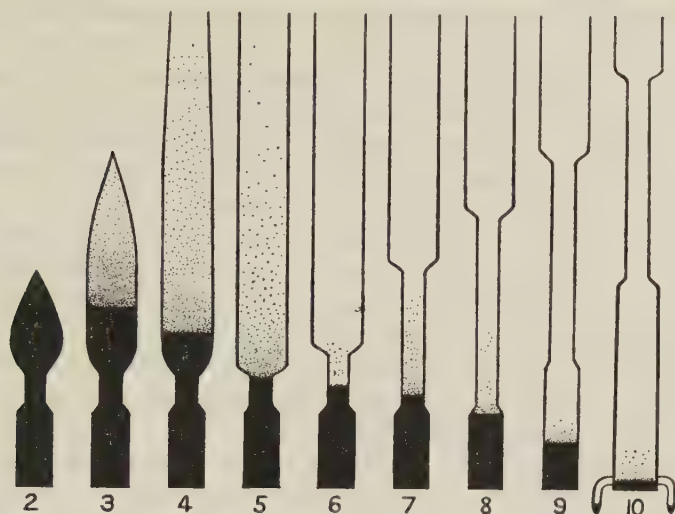
This difference between the top and bottom of the internode has given rise to the idea that there is a basal intercalary meristem, but this receives no



TEXT-FIG. 21. Transverse sections of the same lateral bundle in a partly exposed lamina, showing the basipetal differentiation of the last protoxylem, the metaxylem, the metaphloem, and the sclerenchyma at the positions in Table II indicated by *a*, *b*, and *c* respectively. ( $\times 300$  approx.)

support from the examination of longitudinal sections. The basal parenchyma is now quite large-celled and does not look like a true meristem, although of course its cells are still dividing. The last elongation of the base of the internode is mainly due to the last divisions and the vacuolation and maturation of the cells already present.

Whilst the main and final wave of development of the bundles in the unit



TEXT-FIG. 22. Diagram illustrating the wave of elongation and maturation passing down the lamina, sheath, and associated internode. Solid black indicates the more actively dividing, partially parenchymatized tissue.

composed of the lamina, sheath, and associated internode is thus clearly basipetal, if the trace strands of a young leaf which has not yet entered on this final phase, e.g. the fourth from the apex (Text-fig. 19, *d-f*; see also the lateral illustrated in Text-fig. 18), are followed down into the axis, 2 or 3 internodes below its insertion, they are found to have already present in them expanded flanking vessels and a considerable amount of phloem (Text-fig. 19, *d-f*, and Text-fig. 20). Furthermore the whole of the phloem in this lower part of the course appears to be developed early and to be composed entirely of protophloem. This early basifugal differentiation in the lower regions of the strands appears to be independent of the basipetal differentiation of the upper part which is initiated later in the leaf-tip.

#### *Summary of anatomical development.*

*Plastochrone 'o'.* Primordium only represented by crescent of dermatogen and sub-epidermal divisions.

*Plastochrone 1.* Uppermost primordium about 12 cells long and  $\frac{3}{4}$  encircling the axis. Cell divisions in all planes, but the tip most meristematic. Provascular strand differentiating up axis and towards primordium. This strand has protophloem already developed in lower part of its course.

*Plastochrone 2.* Primordium completely encircling axis and growing by marginal meristems. Median provascular strand now in primordium and 1st lateral provascular strands appear in base.

*Plastochrone 3.* Edges of primordium overlap at insertion. Marginal meristems still active: tip ceases to be meristematic. Protophloem appears in median strand towards the base and differentiates upwards.

*Plastochrone 4.* Marginal meristems replaced by more parenchymatous tissue. Protophloem differentiating up the earlier lateral strands of leaf. First protoxylem may be found in leaf, but sometimes appears in 3rd plastochrone, sometimes not until the 5th. First signs of the axillary bud as a raised structure, but in some apices its appearance is delayed a little. Commencement of the formation of vertical files of cells in lower half of disc of insertion of primordium in the position of the future internode.

*Plastochrone 5.* Protophloem in lateral bundles usually reaches leaf tip. Coincidentally, basipetal provascular strands begin to differentiate down leaf between laterals, reaching base of sheath before end of plastochrone. Cross bundles formed between basipetals, main, and laterals. Ligule formed from epidermal cells on adaxial side of leaf. Leaves separating as internode formation continues. Axillary bud has 1-2 primordia. Leaf apex may be exposed from within the enveloping lower leaves.

*Plastochrone 6.* Basipetal formation of (1) last protoxylem, (2) metaxylem vessels, (3) metaphloem, and (4) metaxylem tracheids. Final elongation of leaf, accompanied by maturation of constituent tissues, in a basipetal wave initiated at leaf apex. Lamina fully expanded and exposed as far as the ligule. Intermediate provascular strands beginning to develop down the internode in the sub-peripheral region.

*Plastochrone 7.* Final expansion of sheath. Vigorous transverse division of cells in axis. First basipetal strands reach base of internode: some may develop towards central regions, forming commencement of anastomosis.

*Plastochrone 8.* Internode extends rapidly and upper part becomes firm owing to maturation of epidermis and sub-epidermal tissues. Basipetal strands have become crowded in sub-peripheral region: at the base of the internode a sub-peripheral meristematic ring is formed.

In seedlings roots now grow out vigorously. In more adult plants the internode appears to continue extension until about the 12th plastochrone, and since the roots wait until this is finished, none grows out until some 5-6 plastochrones after the complete exposure of the lamina below which they are situated, i.e. at about plastochrone 12. Thus in older plants there is a gradually increasing interval between the time when the leaf is fully developed and the time when the roots appear from the node below.

#### DISCUSSION

*Food movement and development.* The youngest primordium, from soon after its inception, has been found to be linked by a provascular strand with



one of the more centrally placed lateral strands of a leaf inserted some internodes below. The more mature the plant, the more leaves there are separating the newly emerging primordium from the leaf to which it is connected. Thus this young primordium is coupled with a leaf below which is fully exposed and presumably no longer 'parasitic' on, but contributing 'food' to, the parent, using the term food in a wide sense. Furthermore, protophloem is developing basifugally up the connecting strand from this contributing leaf before the new primordium has emerged and even before the top of the strand has made actual entry into the primordium. This phloem production in the lower regions of the trace results in the complete differentiation of the tissue on the phloem side of the strand, so that later, when metaphloem differentiates down the strand from the leaf, no new tissue is added here to the already existing phloem. Late basipetal activity is thus confined to the upper part of the course and probably only to that part of the strand where it is in the leaf and the associated internode.

Since, in the lower part of its course, every median-trace strand runs in the sub-peripheral region for a number of internodes, when the meristematic rings develop at the base of the internodes they make contact with it so that connexions are established between the median-trace strand and the backwardly differentiating intermediates of the leaves from nodes successively higher up the axis. Still higher in the axis, where the median-trace strand occupies a more central position, anastomosing strands of the nodal plexus will presumably enable the primordium to tap supplies from still higher leaves.

At the end of the 3rd plastochrone the lateral provascular strands are differentiating up in the leaf and down in the axis, probably linking with others about 6 internodes below (Falkenberg, 1876, and Haga, 1922). During the 4th plastochrone the 1st protophloem, differentiated basifugally, can be found in the early lateral strands, suggesting the movement of food up them. As time goes on, anastomoses will put these lateral strands into continuity with higher and higher leaves.

Thus at first the primordium seems to obtain its food from leaves anything up to eight or more internodes below, the particular number depending on the 'maturity' of the plant. As the young leaf becomes more adult, its supplies probably come in increasing quantities, via the now numerous lateral strands, from leaves progressively nearer to it. Thus its supplies come firstly via the median strand from regions low down in the axis, and then via the laterals from progressively higher levels. In the very young stages, especially before the median strand connects the primordium with other leaves, the general arrangement in vertical files of the cells constituting the axis and the close contact between the cells of the same vertical file may to some extent facilitate food movement.

From the time the leaf is undergoing the final elongation it is becoming progressively less 'parasitic' on the parent plant as more and more of the distal

part becomes mature and begins to contribute food to the shoot. It is at this time that the metaphloem is differentiated basipetally in the median and lateral bundles, while just previous to this the basipetal intermediate bundles were initiated at the top of the leaf and are now penetrating the axial tissues, soon to become fully differentiated to the base of the associated internode and the nodal anastomosis.

After the leaf is adult and all the phloem and intermediate bundles are fully developed, food still has to pass into the stem and to the roots arising at the base of the internode. As the leaf associated with these roots dies, the latter must obtain any further supplies from higher and more distantly situated leaves. Examination of the basal internodes of more mature, flowering plants (i.e. 7–8 internodes below the last living leaves) showed sieve tubes with contents still capable of being stained and presumably capable of translocation.

*Movement of water to expanding leaves.* In the leaves of maize, and presumably those of other monocotyledons, the last-formed xylem, both in the main and lateral bundles as well as in the intermediates, differentiates basipetally. Indeed, except for the first protoxylem elements in the main and lateral bundles, the whole of the xylem of each leaf-internode unit differentiates basipetally. Coupled with the fact that the 1st protoxylem collapses completely during the last extension of the surrounding tissues, this implies that all water going via the vascular bundles from the stem to the leaf lamina must traverse protoplasts, either in the last-formed protoxylem, the metaxylem tracheids, or the flanking vessels. Water entry to the distal parts would thus seem to be entirely under protoplasmic control during a large part of the functional life of the leaf. The distal part of a leaf may thus act as a separate osmotic unit which determines the entry of water from the rest of the plant across the semi-permeable system formed by the protoplasts of the tissues in the zone of last differentiation. The water economy of the leaf during a considerable part of its life may be governed by these factors. In the case of small herbage grasses, when the plants are exposed to the full summer sunshine, and when one would expect the leaves to be experiencing greatest water lack, the reverse may actually be the case since the mature parts of the leaves, acting as separate osmotic systems, will then have their highest carbohydrate concentrations. Such a system may explain such behaviour as the readiness of grass leaves to exude water, the limpness of the leaves of seedlings grown in the dark, and the extremely rapid death of half-exposed leaves when plants which have been growing in the light are placed in the dark.

*Initiation of the median provascular strand.* It is not possible to state the position of initiation of the median provascular strand, since neither the final course nor the early stages seen determine the point of origin. It is probably initiated independently of the rest of the vascular system, originating some little distance down the axis below the disc of insertion of the primordium and differentiating upwards into the new primordium and downwards lower in the axis to unite, at some level determined by internal conditions, with a lateral

running down from an older leaf. This view is supported by the condition reported from the median and lateral strands of *Dactylis*, both of which originate in the disc of insertion of the primordium (Bugnon, P. (1924)), and the median and lateral strands of *Deschampsia* and *Melica*, where the origin is reported to be in the axis below the disc of insertion of the primordium (Philipson, W. R. (1935)). This independent mode of origin must obtain in the axillary buds, where the median provascular strands of the first few leaves must develop basipetally at their lower ends if they are to enter the axis at all. The conclusion that the median strands originate in this way is strengthened by consideration of the independent origin and mode of development of the basipetal strands.

The basifugal nature of the differentiation of the upper part of the course of the median provascular strand seems to be opposed to the idea of Louis (1935) that the procambial strands of vegetative organs originate in or near the 'soubassement foliaire' and to the thesis of Grégoire (1938) that in the case of floral organs the procambial strands are more or less unique in that they originate more or less independently in the axis and extend acropetally into the floral organs.

*Cambium.* There is considerable doubt as to whether true *intrafascicular* cambium has been recognized in monocotyledon bundles, but, in grasses at least, there is some evidence favouring the view that there is an ephemeral *intrafascicular* cambium.

Arber (1917, 1918, 1919, 1922), in a series of papers, brought to a focus much of the evidence. She pointed out the large number of species drawn from many diverse families in which radial seriation could be found in transverse section in the phloem or xylem or in both. She assumed that this radial seriation was the result of the elements having arisen from initials cut off from the cambium. Besides the arrangement of the phloem and xylem, she and others before her have drawn attention to the flattened shape of the cells between these tissues as seen in transverse section.

Radial seriation of the phloem and xylem, together with the flattened shape of the intervening 'parenchyma', can be found in practically every transverse section of the maize axis, especially if the section is near the base of the internode. In more mature parts of the axis a layer of flattened parenchyma may be seen between the xylem and phloem, especially in the median bundles in the centre of the stem, suggesting that the cambium, if any, has vacuolated as the axis matured. Often the last phloem elements and the last xylem tracheids are in radial seriation across this parenchyma.

If the xylem and phloem are in radial rows, this does not prove that they have necessarily arisen from a cambium, meaning by 'a cambium' a layer or a tissue cutting off elements to give xylem on one side and phloem on the other. As for the flattened 'cambial' shape of the cells between the xylem and the phloem, this, as Stover (1934) points out, might easily be due to pressure from the maturing elements of the bundle. He cites an example in which



lateral pressure from a developing vessel has removed all trace of any cambial appearance.

Working on *Asparagus*, Bucur (1936) examined the same bundle at different levels in the stem, assuming that, by comparing the upper part with the more mature lower regions he could form an idea of the manner in which the elements were cut off. He came to the conclusion that the xylem at least arises from a tissue resembling a cambium.

In the present work transverse sections of equivalent bundles to leaves of different ages were examined at equivalent positions in their courses. It is obvious from Text-fig. 16, *d-f*, that the tissue between the first-formed xylem and phloem is insufficient to account for all the elements seen in the mature bundle. Radial increase of the tissue between the first-formed xylem and phloem could be accomplished in either of two ways: (1) The cells may divide horizontally, and some form of adjustment growth occur during the final extension of the internode, causing the two daughter cells to lie side by side radially as far as the greater part of their length is concerned. (2) The cells may divide by longitudinal tangential divisions as in a cambium.

If the cells divide in the second way, the last-formed xylem or phloem elements should have their end walls on the same horizontal level as those of the still undifferentiated cells. But the original provascular strand is itself composed of cells all arranged in such a manner that the end walls are coincidental, giving the strand a stratified appearance in *tangential* as well as in radial view. Thus the end walls of the 1st protophloem cells, the remaining provascular cells, and the 1st protoxylem are inevitably coincidental. It is impossible to find such coincidence in adult internodes, but it can often be seen in the developing region, especially in the nodes where the bundles are not suffering great longitudinal extension. In thick sections of 10–15  $\mu$ , especially if cleared in Eau de Javelle before staining, it will often be seen that the end walls of the xylem or the phloem elements are on the same level as the cells of the remaining undifferentiated tissue. Since this can be seen in strands which contain more cells radially than the original strands possessed, the cells separating the protophloem and protoxylem must have divided tangentially. Often there are indications that horizontal divisions also occur, thus keeping the central cells about the same original length during the longitudinal extension of the strand.

Thus it would seem that the later part of the xylem and phloem must differentiate from cells derived from a cambial zone. Although the cells from this zone for the most part undergo typical cambial tangential divisions, the tissue is too ephemeral for the production of anything approaching a cambial layer. It is as though the remaining cells of the cambial zone vacuolate to parenchyma early and so leave nothing in the nature of a single layer of meristematic cells to cut off future elements.

*Growth at the primordium tip.* In maize a meristem was recorded as active at the primordium tip for about the first two plastochrones. This is also true



for *Dactylis* and *Melica* (Bugnon (1924)) and some twenty other species examined. In other monocotyledons apical growth was observed in *Orchis mascula*, *Arum maculatum*, *Alisma Plantago*, and *Sagittaria sagittifolia*. Growth at the primordium tip is perhaps more common than is realized and may perhaps be responsible for some of the many leaf-tip structures met among the monocotyledons. The maize leaf ends in a short, solid tip; this terminal structure is quite inconspicuous in maize, especially since the end of the leaf soon withers. Arber (1920-2) mentions some thirty or more monocotyledons which have solid tips, small cups, hoods, spines or prolongations at the end of the leaf. They may result from the continued development of the primordium tip growing above the shoot apex where it is comparatively free from any moulding influence due to pressure from the surrounding leaves. Some such similar explanation may apply to the solid tips of *Tamus* and *Dioscorea*, as well as to the precursory appendages of bananas (Skutch, A. F. (1930)).

*Morphology of the shoot apex in grasses.* The slightly pointed apex of *Zea* is not typical of the grasses as a whole. Reference to Bugnon (1924) showed long apices for *Dactylis glomerata* and *Melica altissima* which bore about 6 and 12 primordia respectively on the apical cones, the 6th and 12th respectively being just sufficiently long to have their tips level with the top of the apex. At first it was thought that the apices illustrated might have been elongating considerably just prior to inflorescence formation, but a number of apices of *Dactylis*, sectioned from material collected from the base of plants from October to February, together with a considerable number of dissections at various times throughout the year, showed conditions exactly as described by Bugnon. Furthermore, axillary buds in these sections showed the same elongated type of apex.

Rösler (1928) and Kliem (1931) showed the apices of *Triticum*, *Avena*, and *Secale cereale* to be about as pointed as *Zea*.

The extreme length of the typical grass apex was only realized after examining some of the commoner perennial species. In *Glyceria fluitans*, for example, 9-12 primordia are often found in the stages of development between initiation and the later more rapid growth which brings them over the top of the apex. In *Milium effusum* 8-9 such primordia are found, and in *Arrhenatherum avenaceum* there are about 5.

Thus it would seem that the apex of *Zea* is far from being atypically elongated, but is considerably shorter than in many grasses. It would seem that the grass apices fall into two distinct groups: (a) the long type as found in *Dactylis*, *Melica*, *Glyceria*, *Milium*, &c., (b) the relatively short type occurring in *Zea*, *Coix*, *Avena*, *Triticum*, *Hordeum*, &c. The distinction does not seem to be correlated with the annual or perennial habit, as some annuals (*Poa annua* amongst others) have long apices, while some perennials (e.g. *Saccharum*) have the short type (see Porterfield, 1930, for *Bambusa*).

The characteristics of the grass apex would seem to be: (1) the maintenance of a large amount of relatively unvacuolated tissue in the form of an apical

cone and young primordia, (2) the production of a large number of leaves in various stages, ranging from mere protuberances onwards, i.e. the formation of a 'bud' containing many very young leaf primordia.

The longer the apex, the more does the central tissue tend to become 'parenchymatous' and to divide more and more exclusively at right angles to the long axis of the apex. Thus the longer the apex, the more the central tissue, even here, tends to be composed of files of cells.

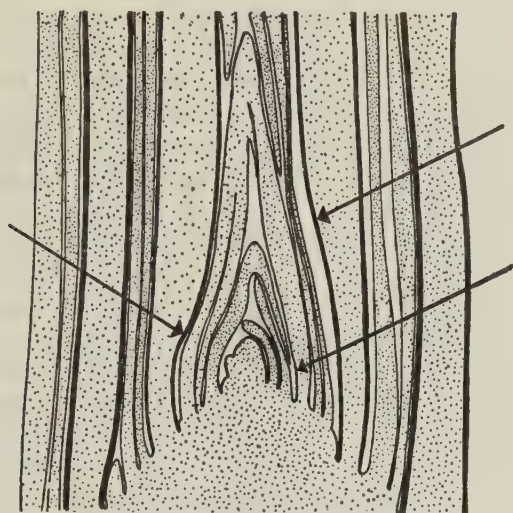
Grass apices of the 'long' type call to mind the aquatic apices of *Elodea* and its dicotyledon counterpart *Hippuris*, both of which have the same curious shape and possess many leaves (though in whorls) in the 'bud'. Here, as in the grass apex, there is the same maintenance of a large amount of relatively unhydrated ('meristematic') tissue clothing a more vacuolated core.

*Closed venation of the leaf.* One of the most characteristic features in the development of the grass leaf is the extremely early production of longitudinal files of cells. By the time the primordium has the first provascular strand the cells of the internal tissue (except for the marginal regions), as well as the dermatogen, are dividing mainly at right angles to the primordium length, leading to the formation of parallel rows of cells. This was particularly striking when dissecting apices of *Zea*, *Dactylis*, and *Glyceria*. The greater width of the more median region is the result of the occurrence of occasional longitudinal divisions, so that there are now two or more files of cells in this part but still only one at the top and bottom. When the lateral strands are initiated, they naturally follow the files of cells and thus diverge in the wider part of the lamina and converge again at the tip, giving the closed venation so typical of the grasses. Later divisions in the tissue between the first laterals provide room for the intermediates. Presumably a very similar course of events holds for many other monocotyledons with a closed venation system.

*Homologies of the lamina and sheath.* Homologies between the lamina and sheath of maize and similar structures in other monocotyledons are not facilitated by this examination of their development. The primordium is extremely undifferentiated at the time when it has just completely encircled the axis (3rd leaf from the apex), and the ligule, which is usually regarded as the boundary mark between sheath and lamina in the adult, arises very late (about the time the primordium is the 5th from the apex) and in some grasses may even be absent. Furthermore, the marginal meristems, when active, are complete and unbroken bands running right down to the insertion, suggesting that, at this stage, there is no real distinction between future lamina and sheath. In two examples of liguleless grasses (*Panicum Crus-galli* and a liguleless variety of maize) it was still easy to distinguish the sheath from the lamina, since the true difference lies in the relative distributions of the lateral and basipetal strands in the two parts. At the base of the lamina there is a progressive union of the basipetals with each other, until in the region of the sheath one basipetal alternates with a lateral. In most grasses the greater part of this union takes place over a short distance and in the region of the ligule,

but in *Neostaphia colusana* R. F. Hoover (*Anthocloa colusana* (Davy) Scribn.) a liguleless grass where the progressive unions are not marked and where they take place over a distance, it is absolutely impossible to divide the leaf into lamina and sheath.

In the developing maize primordium the sheath cannot be distinguished



TEXT-FIG. 23. Position of origin of the ligule with relation to the apex and the surrounding leaves. The arrows indicate the positions of the ligules just below the thicker parts of successive leaves. ( $\times 70$  approx.)

from the lamina until the basipetals have entered it. This region of the leaf has been more compressed and has not formed such a width of tissue between the lateral strands; thus when the basipetals reach this part they unite until only one lies in the tissue between each pair of laterals. This region of union, which is at about the position of the future ligule, is the lowest position above which the young leaf can grow unhindered; below this region the primordium is being compressed between the expanding axis and the leaves outside. The relationship between the position of ligule initiation and the shoot apex is shown in Text-fig. 23.

Thus, the lamina, in a grass at any rate, is a developmental character, probably only equivalent to that part of the leaf which is comparatively unhindered in development; the lamina and sheath probably bear little or no relationship to the part of the primordium which completely surrounds the axis in the early stages. Lamina and sheath are not strict morphological entities, and the structure termed 'sheath' in maize is not necessarily homologous with the sheath of other monocotyledons. Even in the grasses themselves the drawing of homologies as between the lemma and the sheath, and the awn and the lamina (Hackel (1896), also Philipson, W. R. (1934)) may be dangerous.



## SUMMARY

Each leaf primordium is initiated by periclinal divisions in the 'dermatogen', accompanied by divisions in the underlying cells: the rapid 'lateral spread' of these divisions causes the encircling insertion of the primordium. Marginal meristems continue the growth of the primordium and may perhaps be concerned in the formation of the bud which will later appear in the axil of the leaf below. The internode is derived from the lower half of the 'disc of insertion' of the primordium, by repeated horizontal divisions of the cells here, leading to the formation of a tissue of vertical files of cells, each capable of considerable elongation. The ligule arises solely from the dermatogen.

The median provascular strand probably has its origin independent of the rest of the vascular system and at a point in the axis some distance below the insertion of the primordium; in the upper part of its course it is initiated basifugally and reaches and enters the primordium during the first plastochrone. The lateral strands differentiate basifugally in the leaf but basipetally in the axis, having their origin either in the leaf base or in the axis at the insertion of the primordium. Later, basipetal strands appear at the leaf-tip and develop down the leaf and internode, at the base of which they give rise to a nodal network.

Final differentiation of each leaf-internode unit commences at the leaf-tip and passes progressively down the lamina and sheath and finally down the internode, where it is followed by the production of vigorous adventitious roots. In the vascular bundles of the leaf-internode unit, the last protoxylem, all the metaxylem, and all the metaphloem are differentiated basipetally, commencing in the leaf-tip. Where the median and lateral bundles are running in the axis below their leaf-internode unit, their development seems to be more or less uncontrolled by the events occurring in the leaf-internode unit to which they run.

It is suggested that this basipetal wave of differentiation may play a considerable part in the movement of food and water in the maize plant.

There seems to be a definite, if short lived, cambial zone in the median and lateral bundles.

The closed venation of the leaf is an expression of the mode of division of the cells during the early history of the primordium. The demarcation of the leaf into lamina and sheath may perhaps be correlated with the mechanical conditions under which the leaf is developing.

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EXPLANATION OF PLATE VII

Illustrating Dr. Sharman's article on 'Developmental Anatomy of the Shoot of *Zea mays L.*'

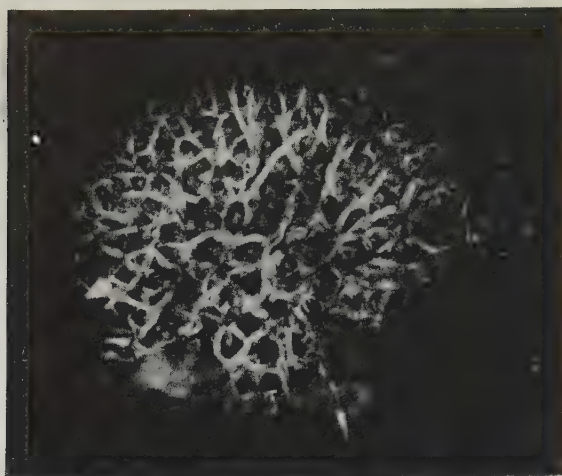
FIG. 1. *Zea mays L.* Adult stem which has been 'retted' after being cut just above the 'node' to show anastomosing strands at the internode base.

FIG. 2. Adult stem split longitudinally and then 'retted' to show the general distribution of the bundles.

FIG. 3. Longitudinal section of the apex. A, relatively larger central cells; B, sub-epidermal cells; C, 'dermatogen' divisions at seat of new primordium; D, insertion of 1st primordium spreading round from the other side of the axis; E, meristematic tip of 1st primordium. ( $\times 250$  approx.)

FIG. 4. Transverse section of the axis just below the insertion of a primordium, showing the well-developed radial rows of cells which foreshadow the future bud. ( $\times 250$  approx.)



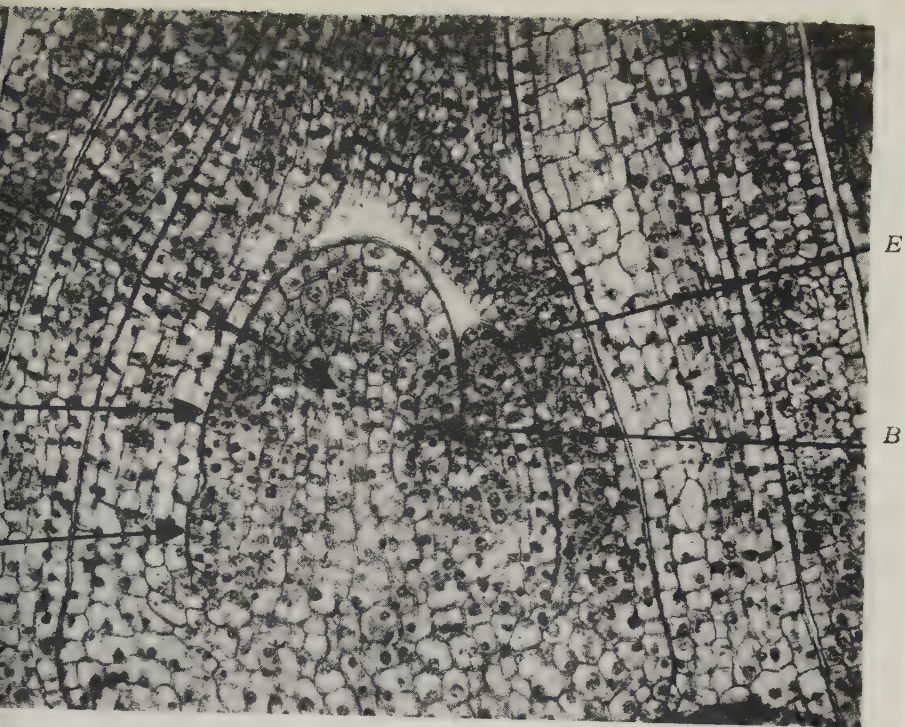


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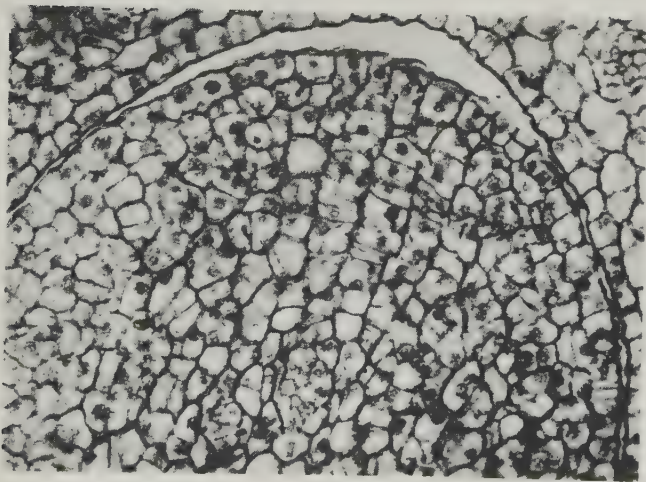


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# A Note on the Cytology of *Psilotum* with Special Reference to Vascular Prothalli from Rangitoto Island

BY

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With Plate VIII and one Figure in the Text

THE object of the present note is to add some cytological facts to the description of the prothalli of *Psilotum triquetrum* Swartz<sup>1</sup> recently given by Holloway (1938, 1939). The essential material, consisting of prothalli and parts of a sporophyte from Rangitoto Island, Auckland, New Zealand, was made available to me by the kindness of Dr. Holloway, after completion of his own morphological work. For comparative purposes this has been supplemented by a limited amount of wild sporophytic material from other sources, and by observations on such living plants as are at present in cultivation in this country. This additional material, while too incomplete to allow of a full study of the genus, is nevertheless sufficient to establish the nature of Dr. Holloway's prothalli with reasonable certainty. In view of the remarkable anatomical features which these prothalli display any definite result would appear to justify publication, even though full elucidation of the subject must await a more favourable occasion.

It may be profitable to recall that *Psilotum* is a tropical genus of supposedly primitive vascular cryptogams consisting at present of only two species, *P. triquetrum* Sw.<sup>1</sup> and *P. flaccidum* Wall. The sporophytes of both species are of world-wide distribution, *P. triquetrum* being the commoner. The gametophytes, on the other hand, have only been discovered quite recently and with great difficulty. They are subterranean branched cylindrical structures, bearing a superficial resemblance to small pieces of rhizome and living saprophytically with the aid of an endophytic fungus. The prothalli of *P. flaccidum* have not yet been seen but there are two records for *P. triquetrum*, namely those of Darnell-Smith (1917) and Lawson (1917) for a locality near Sydney, Australia, and of Holloway (1938, 1939) for Rangitoto Island. The Rangitoto material is of importance in that it has supplied the only known examples of young embryos in archegonia. It also supplied the very remarkable vascular prothalli already referred to. These were all of outstandingly large size and were therefore presumably of considerable age, but

<sup>1</sup> According to the principle of priority the correct name of this species is now considered to be *P. nudum* (L.) Beauv. Since, however, this name is used by only one (Okabe, 1929) of the numerous cytological writers on the plant, the more familiar *P. triquetrum* will be used here.

they were not otherwise abnormal since they bore the usual crop of sex organs. That the sex organs were fully functional was proved by the presence of three specimens in which an archegonium contained an embryo. The prothallial vascular tissue was in the form of a somewhat discontinuous central strand of elongated thin-walled cells surrounded, in the best developed instances, by an endodermis with a Casparian strip. Immediately outside this were commonly found some cells with a conspicuous phlobaphene deposit, and in sixteen out of thirty specimens the centre of the strand contained lignified tracheids from one to three in number when seen in cross-section. Specimens of this kind were obtained by Dr. Holloway on each of two visits made at different times to different parts of the island, and the possible explanations of their nature were summed up by him (1939, p. 335) as follows: 'The occurrence of a vascular conducting tract in the largest gametophytes of *Psilotum* may, from different points of view, be interpreted as due either to some abnormal nuclear condition in such gametophytes, or to physiological changes taking place in the gametophyte as it grows in size, or to the persistence in it of an archaic feature.' This quotation will perhaps be sufficient to explain the special points of interest in the present inquiry.

The genus *Psilotum* has provided cytological material for qualitative study for nearly a century and the earliest literature in which it figures, e.g. Hofmeister (1867), Strasburger (1875, 1880, &c.), Guignard (1894), Farmer and Moore (1904), &c., is fundamental to the beginnings of the science. Nevertheless there are no observations on *P. flaccidum* nor on wild material of known origin of *P. triquetrum*, and attempts at exact quantitative data even for the latter species are both few and uncertain in detail. The reasons for this uncertainty lie in the technical difficulty inherent in many groups of the Pteridophyta. The exact details of the correct haploid chromosome number are still not established; nevertheless the three attempts at enumeration which exist, together with my own observations recorded in Pl. VIII, are in sufficiently close agreement for the present purpose. The first<sup>1</sup> recorded chromosome count, that of Rosen (1896), in what is undoubtedly a very fine piece of work for its date, gave the haploid number as approximately 50 and the diploid as approximately 100. A short paper by Meyer (1928), published in Russian, purports to have confirmed these figures without adding anything to them. Finally Okabe (1929) made a careful study of some horticultural strains in Japan and gave his result, with slight reservations, as 52 for the haploid. Some of Okabe's plants, however, showed twice this number of groups at meiosis. These sporophytes, which he interpreted as tetraploids, were normal morphologically but showed the peculiar feature, affecting about 80 per cent. of the spore mother cells, of a tripolar spindle at the first meiotic division, with the conse-

<sup>1</sup> It is interesting to note that in the very early drawing published by Hofmeister (1867) approximately 50 bodies are shown at the first meiotic metaphase in living material. This is an astonishingly accurate piece of observation since at that time there was no knowledge as to either the nature or the importance of the bodies in question.



quent production of three nuclei instead of two at interkinesis, and of six nuclei instead of four in the 'tetrads'. Other abnormalities were also encountered, but the ripe spores were surprisingly normal in appearance, though nothing was, of course, known as to their powers of germination.

A glance at Pl. VIII, Figs. 1 and 2, will confirm the essential correctness of the chromosome numbers quoted above. Fig. 1 represents the first meiotic



TEXT-FIG. *Psilotum flaccidum* Wall. Chromosomes at the first meiotic metaphase in an aceto-carmines squash, from the cell photographed in Pl. VIII, Fig. 1. The groups drawn in outline are those visible in the photograph, those drawn in solid black are out of the focal plane of the photograph. The diagram was made upon a faint enlargement of the photograph which was used as a camera lucida impression and then bleached away when the drawing was complete. Magnification of the diagram  $\times 3,000$ .  $n$  = not less than 52 nor more than 54.

metaphase in a plant of *P. flaccidum* (no. 739.35 Leiden), of unknown wild origin, growing in the Royal Botanic Gardens, Kew. The interpretation of this figure as representing not less than 52 nor more than 54 pairs of chromosomes is given in the text-fig. The haploid chromosome number of *P. flaccidum* is thus of the same order as that previously recorded for the other species. The diploid form of *P. triquetrum* does not now, unfortunately, appear to be in cultivation in this country; it has therefore not been possible to observe its haploid number directly. Pl. VIII, Fig. 2, represents a late diakinesis in the new specimen H.B. 345, also of unknown wild origin. This is clearly comparable to Okabe's tetraploid since it has rather small paired chromosomes of the order of 200. Some quadrivalent groups are almost certainly present, the most striking examples being marked by arrows, and in view of this it is not possible to obtain an exact count without the simpler form for comparison. Nevertheless the evidence is sufficient to confirm the fact that, as previously reported,

polyploidy exists among the specimens of *P. triquetrum* in cultivation in green-houses, and that the chromosome numbers for the genus as a whole are of the order of 50 for the haploid, 100 for the diploid, and 200 for the sporophytic chromosome number of the tetraploid.

With these facts established the attempt was made to determine the nature of the Rangitoto material. All of this had been fixed in 70 per cent. alcohol and was received from New Zealand in that liquid. A perfect cytological result was hardly to be expected, but after embedding and staining in haematoxylin or gentian violet, the state of preservation was in many cases found to be surprisingly good. This can perhaps be seen from Pl. VIII, Fig 5, which shows a side view of a dividing cell from near the apex of a large prothallus. This prothallus in its older portions possessed a well-marked conducting strand delimited by a layer of phlobaphene-bearing cells, though without actual tracheids. A side view is not itself suitable for determination of chromosome number, but it was found impracticable to give the numerical evidence photographically. Actual counts of a rough kind were, however, made in three other cells of this prothallus. In one nucleus, a late prophase giving the clearest view, the number arrived at was approximately '108', whilst polar views of a metaphase and an anaphase gave '100' and '94' respectively.<sup>1</sup> In another large vascular prothallus two counts obtained were '103' for a polar view of an anaphase and 'not less than 87' for a prophase. Comparable evidence for the small non-vascular prothalli was more difficult to obtain owing to the smaller size of the meristematic areas and the consequent scarcity of dividing cells. One very vigorous small specimen, however, showed a number of well-stained mitotic stages the best of which for the present purpose was a late prophase cut into two unequal pieces. The smaller of the two pieces could be analysed without difficulty and it contained 40 chromosomes and some cut fragments. The larger piece could not be fully analysed, but it certainly contained more than this number of chromosomes. The cell could not, therefore, have possessed fewer than 100 chromosomes.

This somewhat meagre information from the small prothalli could fortunately be supplemented by evidence of another kind. It is now generally recognized that in nuclei of a 'solid' type (cf. Manton, 1935) a definite relation exists between chromosome number and the maximum number of nucleoli to be found in one cell. This relation depends upon the fact, first made clear by Heitz (1931), that in many plants the nucleoli are laid down at telophase in relation to certain parts of specific chromosomes. After formation their number may be reduced by coalescence, since the nucleolar substance is liquid, but the maximum number formed is determined by the number of nucleolus-forming chromosomes present. It is fortunate that many of the early workers

<sup>1</sup> The use of inverted commas for the numerical results is intended to emphasize that these must be regarded as rough estimates only and not comparable in exactness with what is usually obtainable with more refined methods of fixation. The numbers obtained are more likely to be too low than too high, but they do not represent an accuracy greater than to within 10 or 20 chromosomes of the correct value.

on *Psilotum*, e.g. Karsten (1893), Guignard (1894), Rosen (1896), recorded observations on the nucleoli of their sporophytes. All were agreed that the average number was two or three per cell and Rosen in particular gave three as the commonest number in his sporophyte which is known to have been a diploid. On examining the prothalli it was a matter of considerable interest to find that two or three nucleoli per cell were also the commonest numbers in each of the three prothalli in which chromosomes had been seen, although in a few cells of all three prothalli as many as four nucleoli were encountered. Nucleolar counts of exactly the same kind were also made in two additional small prothalli and in one other large one. All the evidence seems therefore to show that, while the large and the small prothalli are cytologically indistinguishable, none of them is haploid but all are probably diploids.

Demonstration of diploidy in a prothallus does not in itself provide any explanation of the occurrence of anomalous conducting tissue, since many examples of diploid prothalli are known in the Pteridophyta, e.g. *Osmunda* (Manton, 1932), *Aspidium*, *Woodwardia* (Lawton, 1932), and no morphological aberration need accompany a simple doubling of the chromosomes if the parent plant is genetically normal. The fact did appear, however, to justify the expectation that the Rangitoto diploid prothalli would be found to be related to a tetraploid sporophyte. To determine this point Dr. Holloway very kindly supplied the sporophytic material, consisting of pieces of rhizome and some young fertile twigs originally fixed on the island.

Pl. VIII, Fig. 6, shows a side view of a mitotic figure in a rhizome apex from the Rangitoto sporophyte. The material, also fixed in 70 per cent. alcohol, was more difficult to handle than the prothalli and the quality less good. Nevertheless, the cells can be seen at a glance to be larger (compare Pl. VIII, Fig. 6, with Pl. VIII, Fig. 5), the resting nuclei contain 6, 7, but never more than 8 nucleoli, while in the best mitosis seen, an oblique polar view cut into three pieces, the count obtained was 'more than 170'. This sporophyte appears, therefore, to be a tetraploid as expected.

Additional information was obtained by examination of the fertile twigs. These (see Pl. VIII, Fig. 7) bore sporangia in all stages of development, and the spore mother cells could be studied both in aceto-carmine and in section with results that are illustrated in Pl. VIII, Figs. 8-13. Without attempting to determine fine details such as the presence or absence of multivalent pairing, the general impression gained is of a grossly abnormal meiotic mechanism in a large number, though not quite all, of the cells. The abnormalities, as in the Japanese tetraploid described by Okabe, seem to originate in malformations of the spindle, though the details in the two cases differ slightly. Thus no instances of three equal poles were encountered in the Rangitoto material but many examples of three unequal poles (Pl. VIII, Fig. 10) were met with, which resulted in the formation of three unequal nuclei at interkinesis (Pl. VIII, Fig. 8, above) and of three dissimilar metaphase plates at the second division (Pl. VIII, Fig. 12). Some examples of four unequal nuclei at interkinesis (Pl. VIII, Fig. 9,



right) were most easily explained by postulating aquadripolar spindle, whilst the occasional presence of one large plate or of two grossly unequal metaphase plates (Pl. VIII, Fig. 11) at the second division seemed to suggest a fusion of spindles at that division. At the end of meiosis 'tetrads' with anything from two to about ten nuclei were encountered, six nuclei being the commonest number (Pl. VIII, Fig. 13, left). Nevertheless, a few normal mother cells and tetrads could always be found (Pl. VIII, Fig. 9, left; Pl. VIII, Fig. 13, centre) which, in sections, could be traced to a few tapetal pockets which were unmixed with abnormal cells. Abnormal cells were, however, greatly in the majority, the proportion actually estimated in one sporangium being 70 per cent. In spite of this high frequency of gross irregularity the external morphology of the ripe spores was surprisingly undisturbed, though some spores could be seen to have two nuclei.

Aberrations of this type, caused by the persistent presence of multipolar spindles, are on the whole so unusual among plants that a causal explanation cannot at once be arrived at. As pointed out by Okabe and others, e.g. Heitz (1926), tripolar spindles have only previously been met with as isolated cells in certain hybrids and a few pure species, e.g. *Melandrium album* Heitz (1926), or they have been induced on a larger scale as a pathological response to violent external influences such as sudden exposure to cold (e.g. Michaelis, 1926, on *Epilobium*). I have myself obtained such spindles on one occasion in an otherwise normal tetraploid *Osmunda* as an apparent consequence of the very unusual succession of weather in 1939-40 when a phenomenally cold winter was succeeded by a very hot and dry spring. The case of *Psilotum*, however, was, and remains, unique in that tripolar spindles occur in great abundance for no apparent reason. Whether the effective cause will be found in reality to be environmental or to be due to some specific genetical peculiarity can only be determined by experiment. There is, however, no precedent for assuming it to be a necessary consequence of the mere fact of tetraploidy.

It would therefore appear that both generations of the Rangitoto strain of *Psilotum* possess anomalous characteristics which cannot be explained merely by reference to their polyploid condition. The anomalous characteristics are the abnormal spindle at meiosis in the sporophyte and the peculiar vascular tissue in the gametophyte. Whether there is any causal connexion between these characters cannot at the moment be determined. It is possible that a connexion may exist, for a meiosis of the type described could, in theory at least, introduce an unlimited amount of genetical disturbance, in the form of extra chromosomes, deficiencies, &c., into the next generation. In that case 'abnormal nuclear condition', the first of Holloway's three alternatives, might well be the true explanation of the prothallial tracheids. Such additional nuclear abnormality cannot, however, be assumed to exist for theoretical reasons alone and it is impossible to detect it directly until more refined cytological treatment can be applied. Moreover, the possibility is equally open that the theoretical consequences of the meiotic behaviour might not in fact be realized. Most, if not



all, of the abnormal spores produced would certainly be non-viable, and since both generations possess ample means of vegetative propagation by gemmae, the entire prothallial population on the island could have been produced from the small number of normal mother cells or from an ancestral plant with a regular meiosis. As long as the possibility remains open that the Rangitoto material may prove to be a simple autopolyploid, the cytology must be regarded as inconclusive in distinguishing between Holloway's alternative explanations of the prothallial structure.

Though further work will be necessary before the solution to this problem will be found, a number of new lines of approach have been indicated by the results arrived at. Since all the prothalli from Rangitoto appear to be diploids and there are no cytological facts for the Sydney material, the most direct possible contribution to the main problem would be the discovery of undoubted haploid prothalli of comparable size and age to the large vascular specimens under discussion. Another valuable line of approach, both for its own sake and perhaps as a prerequisite to the finding of haploid gametophytes, would be the tracing out of the geographical ranges of diploid and tetraploid sporophytes in Nature and the collection of data as to the prevalence, or otherwise, of abnormal meioses in the tetraploids. On the latter point I have nothing further to add since I have no wild sporangial material from any source other than Rangitoto island. Two additional wild specimens with vegetative meristems have, however, come into my hands which are worth quoting as a further indication that natural populations with different chromosome numbers exist in the species. Pl. VIII, Figs. 3 and 3a, show the two opposite focal levels of one nucleus from the tip of a stout young aerial shoot fixed in absolute alcohol at Hakgala, Ceylon, by Professor Lang in 1900, and subsequently preserved in 70 per cent. alcohol. It shows a very beautiful plate of approximately 100 chromosomes that can only belong to a diploid sporophyte. Pl. VIII, Fig. 4, is of one focal level of a cell in a rhizome apex fixed quite recently in half-strength chrom-acetic-formalin<sup>1</sup> in Malay and posted to me in that liquid by Dr. Chapman of Kemendore Estate, Malacca. It is another tetraploid and in this respect the Rangitoto specimen is not unique among wild plants.

Since the full exploration of a subject such as the geographical distribution of a tropical species is unlikely to be completed by one person, I might perhaps express my willingness to contribute to it at any time if collectors or

<sup>1</sup> This reagent is very valuable for use in the field, since material can remain in it indefinitely without damage. It is composed of two stock solutions, A and B, of the following formula. Sol. A: 1 gm.  $\text{CrO}_3$  (chromic acid) + 65 c.c. distilled (or rain) water + 10 c.c. glacial acetic acid. Sol. B: 40 c.c. of fresh commercial formalin + 35 c.c. distilled water. Mix the solutions together in equal parts immediately before use and dilute the mixture with an equal volume of water for the half-strength fixative. The weaker solution is generally preferable for vegetative tissue of the Pteridophyta but the full strength is often required for sporangia. Since the fixative is not very penetrating material must be in small pieces, i.e. as isolated sporangia or detached rhizome apices not more than an eighth of an inch long. For preserving complete twigs 70 per cent. alcohol is the most suitable reagent.

correspondents should be in a position to send material. Any of the very simple methods of fixing and preserving quoted in the text can be made to yield serviceable results provided that the geographical origin of the plant fixed is clearly recorded.

#### SUMMARY

1. The haploid chromosome number of a plant of *P. flaccidum*, of unknown wild origin growing at Kew, is shown to be not less than 52 nor more than 54. A tetraploid specimen of *P. triquetrum* (= *P. nudum* (L.) Beauv.), also of unknown wild origin at Kew, is shown to possess about 200 somewhat smaller chromosomes paired in a manner strongly suggesting the presence of some quadrivalents. The evidence of previous writers is therefore confirmed that polyploidy exists among horticultural specimens of *P. triquetrum*, and that the haploid, diploid, and tetraploid chromosome numbers for the genus are of the order of 50, 100, and 200 respectively.

2. Approximate chromosome counts have been made in three prothalli of *P. triquetrum* supplied by Dr. Holloway from Rangitoto Island, Auckland, New Zealand, two being large specimens with the peculiar central conducting strand originally described by Holloway and one being a small non-vascular specimen. All three appear to be diploids.

3. Additional evidence from nucleolar counts has been obtained from two other small prothalli and one other large one. These three also appear to be diploids.

4. A chromosome count and nucleolar counts made on a rhizome apex from Rangitoto Island, supplied by Dr. Holloway, have shown this sporophyte to be a tetraploid. This is the first tetraploid *Psilotum* for which the geographical origin is known.

5. The sporangia on this specimen show abnormalities of meiosis recalling some previously described for a horticultural tetraploid in Japan (Okabe, 1929).

6. The existence of polyploidy among wild populations of *P. triquetrum* has been further confirmed by the finding of a diploid sporophyte at Hakgala, Ceylon, and another tetraploid in Malay.

7. The cytological facts do not yet justify a decision as to the interpretation to be put upon the presence of conducting tissue in the large gametophytes.

8. It is recommended that further search should be made for a true haploid prothallus and that the geographical distribution and meiotic behaviour of diploid and tetraploid sporophytes should be further investigated.

My thanks are due to the authorities of the Royal Gardens, Kew, for permission to take material for fixation from two species, and most particularly to Dr. Holloway for his very helpful co-operation and for the privilege of allowing me to investigate his unique material. I am also indebted to Professor Lang for kindly criticism, and to him and Dr. Chapman for gifts of supplementary material.

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## EXPLANATION OF PLATE VIII

Illustrating Dr. I. Manton's paper on 'The Cytology of *Psilotum* with Special Reference to Vascular Prothalli from Rangitoto Island'.

(All the figures are from untouched photomicrographs)

Fig. 1. *P. flaccidum* (Kew specimen number 739.35 Leiden). The first meiotic metaphase from an aceto-carmine preparation. Diagram in Text-fig.  $n$  = not less than 52 nor more than 54. ( $\times 1,000$ .)

Fig. 2. *P. triquetrum* (Kew specimen number H.B. 345). Late diakinesis from an aceto-carmine preparation. The arrows indicate probable quadrivalents.  $2n = c. 100$ . ( $\times 1,000$ .)

Fig. 3. *P. triquetrum*. Wild sporophyte from Ceylon fixed in absolute alcohol in 1900. Figs. 3 and 3a show the two opposite focal levels of a mitosis in a stem apex with the diploid chromosome number, from a section stained in haematoxylin. ( $\times 1,000$ .)

Fig. 4. *P. triquetrum*. Wild sporophyte from Malay fixed in half-strength chrom-acetic-formalin, stained in haematoxylin. One polar view of a mitosis in a rhizome apex with the tetraploid chromosome number. ( $\times 1,000$ .)

Fig. 5. *P. triquetrum*. A large vascular prothallus from Rangitoto Island fixed in 70 per cent. alcohol, stained in haematoxylin. The side view of a cell near the apex. The prothallus is diploid. The pear-shaped body below the equatorial plate is a nucleolus which has been deformed by the spindle. ( $\times 1,000$ .)

Fig. 6. *P. triquetrum*. A sporophyte from Rangitoto Island fixed in 70 per cent. alcohol, stained in haematoxylin. The side view of a cell from a rhizome apex. The plant is tetraploid. ( $\times 1,000$ .)

Fig. 7. *P. triquetrum*. External view of a twig from the Rangitoto sporophyte in 70 per cent. alcohol. ( $\times 2$ .)

Fig. 8. The Rangitoto sporophyte. Spore mother cells from an aceto-carmin preparation. Interkinesis, the lower cell losing chromosomes, the upper with three nuclei. ( $\times c. 300$ .)

Fig. 9. Spore mother cells as Fig. 8. A normal metaphase and interkinesis to the left, an interkinesis with four nuclei to the right. ( $\times c. 300$ .)

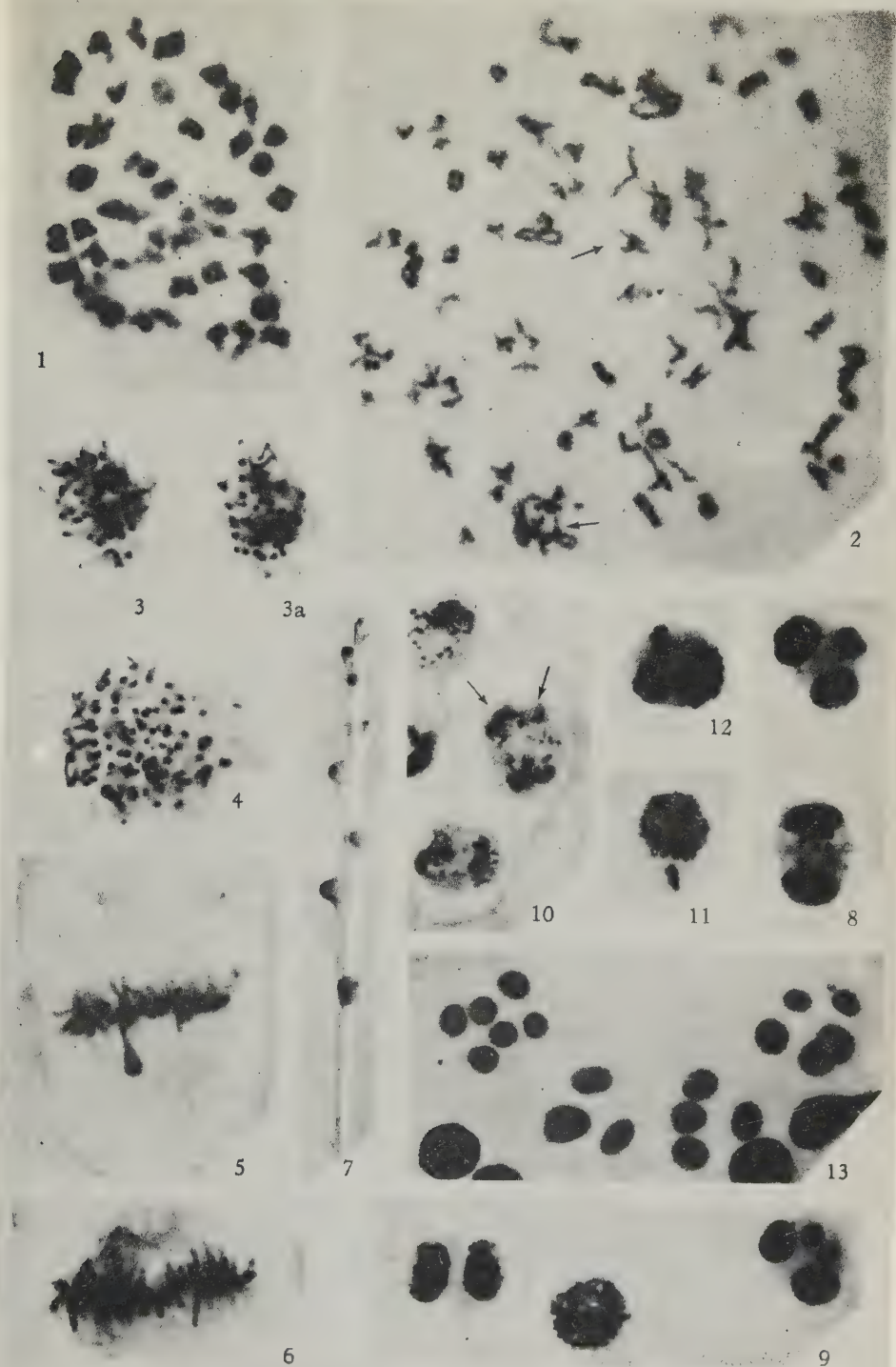
Fig. 10. The Rangitoto sporophyte. Part of a group of mother cells in a pocket of the plasmodial tapetum showing anaphase of the first meiotic division with a tripolar spindle. From a section stained in haematoxylin. ( $\times 500$ .)

Fig. 11. The Rangitoto sporophyte. The second meiotic division from an aceto-carmin preparation. An abnormal cell with two grossly unequal metaphase plates. ( $\times c. 300$ .)

Fig. 12. As Fig. 11. An abnormal cell with three dissimilar metaphase plates. ( $\times c. 300$ .)

Fig. 13. As Fig. 11. Normal tetrads in the centre; abnormal tetrads with extra nuclei left and right. The large single nuclei between the tetrads belong to the tapetum. ( $\times c. 300$ .)





Huth, Stubbs X. Kent.



# The Gaseous Exchange of Seeds and Isolated Cotyledons of *Cucurbita Pepo*

BY

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With eight Figures in the Text

THE present series of experiments was designed to analyse the metabolic changes that occur in the cotyledon during the first 48 hours of growth. The restriction to this limited period was suggested by the results of a previous investigation in which it was shown that the early reactions of the cotyledons have a marked effect on subsequent development (Brown, 1941). Although the primary purpose of this investigation was to obtain data on the metabolic changes induced by different environmental conditions, direct measurement of these by chemical analysis was not attempted; instead it was thought that an indirect evaluation by an analysis of the gaseous exchange would be adequate for the immediate purpose. By these means a number of investigators have analysed very effectively the metabolic changes occurring in the seed.

The main interest throughout this series of experiments is the reaction of the cotyledon in relation to the germination of the seed as a whole. The primary emphasis is not on the independent reactions of the cotyledon but how these affect, and are affected by, the functioning of the whole system of the seed to which they belong. Accordingly, the investigation has two separate aspects, one involving an examination of the gaseous exchange of the seed in its entirety and the other that of the single cotyledon in isolation or attached to other fragments of the seed. The relevant literature therefore covers two separate fields of inquiry, to one of which—that on the gaseous exchange of the whole seed—there have been a very large number of contributions. The gaseous exchange of the single cotyledon whether or not in isolation has, on the other hand, received little attention.

No attempt is made here to review the literature on the gaseous exchange of seeds. Much of it has already been very adequately reviewed by Stiles and Leach (1932, 1933), by Leach and Dent (1934), and by Leach (1936). The contributions of immediate interest in relation to the results of the present investigation are described in the last section of this paper.

## MATERIALS AND METHODS

In an earlier contribution (1941) the conditions that must be fulfilled in obtaining quantitative data relating to cotyledons are discussed at some length.

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The same methods as before were adapted for the purposes of the present experiments. The seeds were soaked in calcium hypochlorite solution for two hours, washed in sterile distilled water, and any further manipulations were then conducted between two glass sheets which were swabbed with alcohol at frequent intervals.

For reasons that are described below it was necessary that the serial observations should be made on the same sample of material. This could be done either by culturing the material in the respiration apparatus or by transference from a culture dish for each experiment. In order to avoid the risks of infection and damage the first of the methods was adopted.

Accordingly, after removal from the seed the experimental material was transferred to a chamber of the respiration apparatus. This was in the form of a cup which held sterile glass beads to which a requisite amount of sterile distilled water had been added. The water held on the surface of the beads came in contact with the respiring tissue; at the same time gases could still diffuse through the interstices between the beads, and a gaseous flow was therefore maintained over the surface in contact.

The variability in every sample of seed is considerable, and the differences in oxygen uptake or carbon dioxide production between any two seeds are therefore liable to be large. For this reason, as in this investigation serial observations were required, the measurements were all made on the same sample of material. Stiles and Leach (1932) have emphasized the value of observations based on single specimens. The value of this provision in this investigation was enhanced by the need for maintaining the material sterile throughout the series of observations made. Cultures containing more than one specimen entailed the risk of infection, and since the rate of this was always fairly high it was necessary to adopt all possible means for reducing failures due to this cause.

The great variability among a group of seeds makes it difficult to compare the reactions of any two seeds when each is subjected to a different set of experimental conditions. A comparison of the effect of a pair of alternative treatments can, however, be made with more precision using the cotyledons from the same seed, which differ in their reactions remarkably little. The policy of using the two cotyledons from the same seed was therefore adopted when comparisons of the effects of two different treatments were being made.

In this paper the results are stated as c.c. of gas absorbed or evolved per cotyledon per hour. Other investigators have frequently used either fresh weight or dry weight as the basis of comparison. The use of these standards is, however, only justified if they provide a measure of the mass of actively metabolizing tissue. There is some doubt as to whether weight is ever a wholly satisfactory standard of reference; in the case of the seed it is clearly unsatisfactory, for development is not characterized by a uniform change throughout the body of the cotyledon. In the early stages change is evident first at the surfaces, and in the case of the attached organ at the base.



Only at a later stage does activity begin in the closely packed cells in the central tissues of the cotyledon. For comparative purposes weight might be justifiable as standard of reference if the proportion of active cells to the whole was constant at any particular stage of development. The cotyledons vary in form, however, and particularly in thickness, and these variations must occasion corresponding variations in the proportion of active and dormant cells. The difficulty was minimized by using only seed of a certain weight and shape, and by expressing the result in terms of the unit cotyledon. This provides data with which comparisons can be made with some confidence, and also has the advantage that in these terms the data serve as an expression of the development of the organ and can be used for the analysis of that process.

In this paper three terms are used which require some explanation. The two surfaces of the cotyledon are described as the inner and the outer surfaces respectively, according to the position which they occupy in the seed. The outer surface is normally covered by the seed-coat membranes, the inner is in contact with the other cotyledon.

The seed-coat of *Cucurbita Pepo* consists of two parts, an outer thick, rigid membrane and an inner thinner and much more pliable one. The gaseous exchange of the seed is powerfully affected by the presence of the seed-coat, and this has been shown to be due almost entirely to the inner membrane (Brown, 1940). Accordingly this membrane figures largely in the present investigation and is referred to as the 'pellicle'.

#### APPARATUS

The apparatus used was designed to fulfil the conditions already outlined, namely, (1) that the experimental material should be cultured in the apparatus in which the gaseous exchange is measured, and (2) that the apparatus should be sufficiently sensitive to measure the gaseous exchange of a single cotyledon; further, the data must include the measurement of the oxygen absorbed as well as that of the carbon dioxide evolved. The importance of these measurements has been stressed by a number of writers, and is indeed sufficiently evident from the results of this investigation. The principle adopted in this work has hitherto been extensively used; namely, measuring in a closed system the change of pressure, if any, occasioned by the gaseous exchange of a tissue, and the further change of pressure after the removal of the carbon dioxide. From these two sets of data the volumes of oxygen absorbed and of carbon dioxide evolved can be calculated. If, however, the gaseous exchange is measured manometrically it is desirable that the internal volume of the system should be kept as low as possible, and under these conditions, unless the atmosphere above the tissue is renewed fairly frequently, the oxygen may quickly become reduced.

The apparatus was therefore designed to enable a circulation of air to be maintained over the cultures in the intervals between the actual measurements.

This provision, however, increased the difficulty of maintaining the experimental material sterile. Attempts were made to sterilize all the glass tubes through which the air was passed and to filter this air before it was drawn into the apparatus. These measures were not successful, largely because of the difficulty of sterilizing without heat a system of narrow glass tubes. Finally, although air was always circulated over the cultures in the intervals between the measurements, the measures designed to keep it sterile were abandoned. The percentage of failures through infection was naturally very high—about 30 per cent. The percentage of failures was not, however, as high as that involved in the only other alternative method. In a preliminary series of experiments, in which cotyledons were transferred from a culture dish for each separate determination, three out of four had to be abandoned because of infection.

The apparatus designed for these experiments is shown in diagrammatic form in Fig. 1. The experimental material is placed in the receptacle A through which a current of air can be maintained by way of the tubes I and J and the three-way taps H and K. For the purpose of measuring the gaseous exchange A is isolated from the atmosphere by manipulating the taps K and H, and after a certain period the volumes of the exchanged gases are determined. When H and K are turned so that A is in communication with C and L respectively, the apparatus consists of two U-tube reservoirs of fluid CDE and LMNOP, one at each end of a closed system which includes, in addition to the respiration chamber A, a tube B containing potassium hydroxide. Clearly the air in such a system can be displaced alternately towards either end by applying positive and negative pressures at the open end of one of the U-tube reservoirs. The carbon dioxide which accumulates in the respiration chamber is in the course of the backward and forward motion of the air forced through the potassium hydroxide tube in which it is absorbed. There is a consequent fall in pressure which, when measured, provides an estimate of the volume of carbon dioxide produced in A.

In the reservoir system CDE a large fluid exchange can be induced between the bulb C and the wide-bore tube E, both of which have a large capacity. Above C the system is continued as a capillary tube on which there is a mark G which stands above a tap F. The reservoir LMNOP involves two subsidiary systems. The two limbs MN constitute a manometer on which the pressure difference occasioned by the absorption of  $\text{CO}_2$  is measured. On this side the fluid exchange during the displacement of the air in the apparatus occurs along the system LMOP and between the bulb L and the composite tube OP, which consists of a lower section O with a large diameter and of a narrower portion P graduated in 100ths of a c.c.; O and P being separated by the tap R. When fluid is released from OP, it is forced to flow only into M and thence into L by closing the tap Q at the upper extremity of N.

Before an estimation of  $\text{CO}_2$  content is to be made the fluid on the side of CDE stands at G with the tap F closed; on the side of LMNOP the fluid in M and N is at

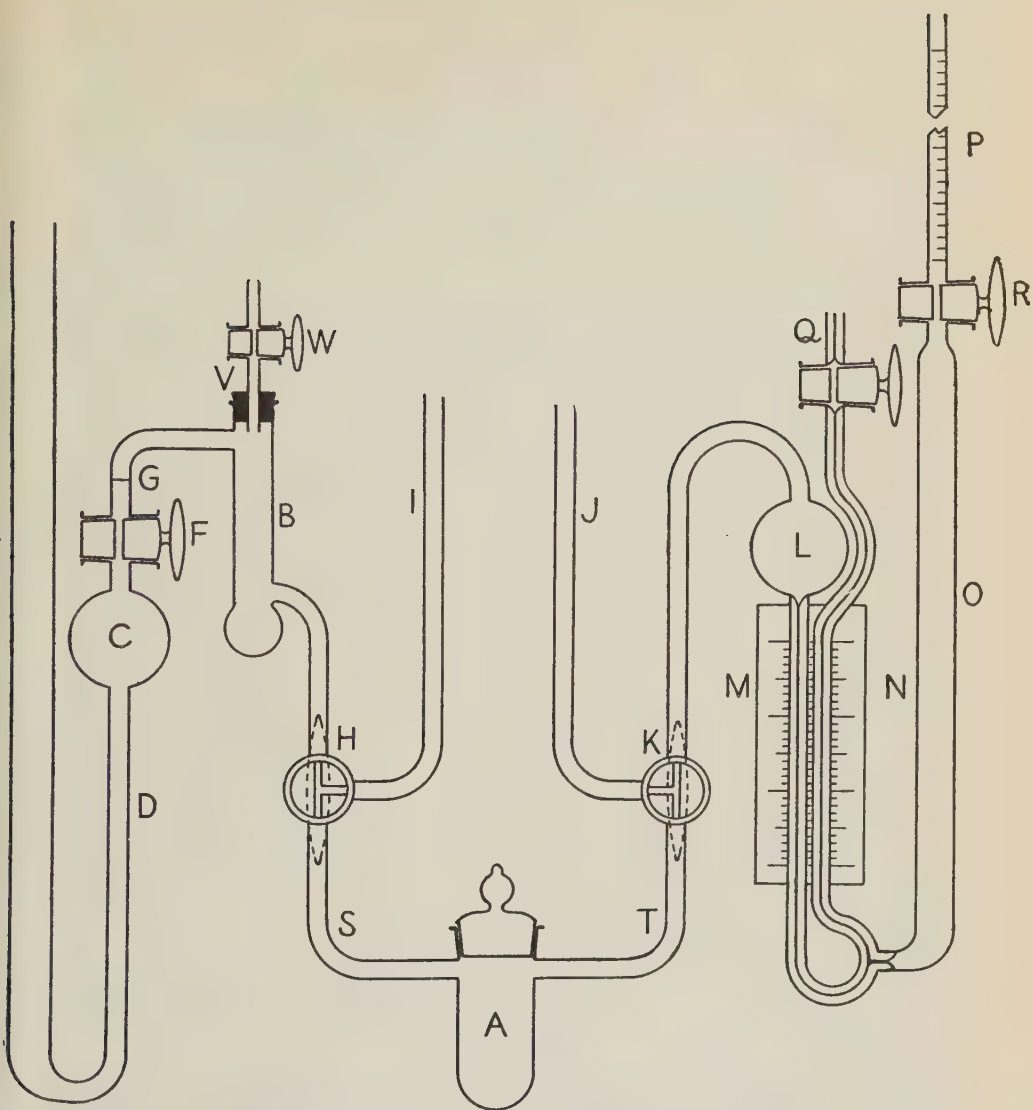


FIG. 1. Apparatus for measurement of gaseous exchange of single cotyledons.  
For explanation see text.

the same level and in P it is at a known level; the tap Q is open and the tap R is closed. In order to estimate the  $\text{CO}_2$  evolved in A the tap Q is closed and R and F are opened. Positive and negative pressures are then alternately applied through a rubber tube to the open end of P. When the absorption of the  $\text{CO}_2$  is complete, the fluid on the side CDE is restored to its original level at G, and F is closed; on the side of LMNOP fluid is drawn into OP until it reaches the original level in P. R is closed and Q is opened. The fluid in M and N is now at different levels, and this difference constitutes a measure of the  $\text{CO}_2$  evolved in A and absorbed in B.

The solid potassium hydroxide in B requires frequent renewal, and it is therefore provided with an open neck through which the contents of the tube can be withdrawn. When the apparatus is in use the stem v of a tap w is secured in the open neck by a rubber sleeve; with tap w closed direct communication of B with the atmosphere is broken.

The pressure change that is registered in the manometer MN can be recorded as such or it can be converted into a volume change. The latter is the more convenient since it avoids the necessity of an elaborate measurement of the internal volume of the apparatus and of the volume of the experimental material which during the course of the experiment is continually changing. By means of the graduated tube P the volume corresponding to the pressure difference indicated is readily determined. With a reduction in pressure the more usual condition—the fluid level rises in M and falls in N. The pressure is adjusted to that of the atmosphere by releasing fluid from P to flow into MN until the fluid in the two arms stands at the same level; the volume thus released from P is clearly double the volume change in the apparatus.

With this apparatus the oxygen uptake can be determined at the same time as the  $\text{CO}_2$  production. Tap H is turned to break communication between A and both B and I. Tap K is turned into the position in which communication is broken between A and J and established between A and bulb L. SATL is then a closed system in which pressure changes are communicated directly to the manometer. Under these conditions any pressure differences shown on the manometer are due to the unequal volumes of oxygen absorbed and carbon dioxide evolved. In this case also the pressure difference is converted into a volume change by the method indicated in the last paragraph. From this observation, the volume of carbon dioxide production being known, that of oxygen absorption is easily calculated.

The technique employed requires, however, that a correction should be applied to the primary data before the volumes of the exchanged gases are calculated. After the determination of the difference between the volumes of the exchanged gases is made, the fluid in M and N is at the same level, and the estimation of the carbon dioxide accumulated during the same period can be undertaken immediately. The backward and forward displacement of fluid involved in this estimation occupies, however, a further 10 minutes, during which time the unequal absorption and evolution of gases is still continuing.



The volume difference due to this factor must therefore, be estimated from the first determination, and corresponding compensation made in the value for the volume of carbon dioxide obtained from the second.

Five possible sources of error inherent in the method have been considered. (1) Errors may arise through inaccurate adjustment of the fluid levels at G, in P, and in the arms of the manometer MN. This error is reduced by making as narrow as possible the tubes in which the adjustments are made. The adjustment to the same level of the fluid in M and N is facilitated by bringing these two limbs together. (2) The potassium hydroxide in B absorbs not only carbon dioxide but also water vapour and this may exaggerate the volume change in the apparatus. In view of the small volume of air this error is likely to be small, and in any case the absorbed vapour is rapidly replaced by further evaporation in the chamber A. Small errors may, however, arise if the replacement is not complete before the manipulations have been made which are necessary for the determination of the volume change. The error from this source is certainly not more than 2-3 per cent. of the final reading and can be disregarded. If necessary a correction can be made by estimating the volume change due to this factor in a blank determination where the manipulations involved are extended over the same period of time as they are in the normal experimental routine. (3) After the withdrawal into OP of fluid from L some of the liquid is left clinging to the walls of L and this, as it drains downward, causes the height of the column in M to increase slowly. The error from this source can be eliminated completely by bringing the fluid in MN to the same level in the manner described above and then setting the tap K to break communication with both J and T. When drainage from L into M is complete a further adjustment from P is made. (4) The changing concentrations of each of the exchanged gases in A must lead to corresponding changes in the concentration of these gases in solution in the water of the culture medium, depending primarily on the proportionate change in the partial pressures of the gases in the atmosphere. The partial pressure of oxygen in the atmosphere being fairly high, the proportionate change in the concentration of this gas is not likely to be large; and in any case the absolute amount of oxygen dissolved in the water can never be large since its solubility is comparatively low. The position is different with carbon dioxide. The proportionate change in the partial pressure of this gas must be large, since its original concentration is low; in addition the solubility of this gas in water is high. It is therefore probable that comparatively large quantities of this gas are released into the atmosphere of the chamber as the concentration of the gas is reduced by absorption in the potassium hydroxide. Moreover, in a system in which the liquid component is not agitated the establishment of a new equilibrium position is likely to be slow. There is a danger, therefore, that some of the carbon dioxide produced by the tissue will dissolve in the water and be released into the atmosphere only after it has been forced through the potassium hydroxide. The resulting error can be eliminated not only by repeating the backward and

forward displacement several times but also by performing these operations in two stages separated by an interval of 5 minutes. (5) No provision is made for possible disturbance due to changing atmospheric pressure. It is probable, however, that the error is not large when the experimental period is short. The period of 30 minutes adopted in these experiments is sufficiently long to ensure an adequate change of pressure due to respiration, and sufficiently short to preclude the probability of any considerable change in atmospheric pressure.

The results described in the next section were obtained with two instruments which were in continuous operation. They were immersed in a water-bath regulated to within  $0.2^{\circ}\text{C}$ . In the intervals between measurements air was drawn over the living tissue. In most of the experiments cotyledons were used as the experimental objects; the cotyledons coming from the same seed were allotted each to one of the pair of instruments. When the cotyledon or other experimental material was in position, the respiration chamber A was closed with a greased, ground-glass stopper. H and K were immediately turned to bring A in communication with the atmosphere through I and J. The apparatus was now transferred to the water-bath and either I or J was then attached to a coil which was also immersed in the water-bath, the other arm being attached to a water pump through a small bubbler acting as a flow-indicator.

The experiment was always started in the late afternoon. The following morning the first measurement of the gaseous exchange was made. Since air had been circulated over the experimental material, it was necessary that either the  $\text{CO}_2$  content of the system should be known or that this gas should be removed from the atmosphere above the respiring material; being more convenient the second alternative was adopted. The method used was that of displacing the air backwards and forwards several times in the manner already described; after which the several preparatory adjustments, necessary for the estimation of the exchanged gases, were made.

Each experiment was usually continued for forty-eight hours, and during this time four measurements of the respiration rate were usually made. At the end of the experiment the cotyledon or other material was removed from the apparatus, pressed between several layers of filter-paper to remove surface moisture, and weighed. After drying overnight in an oven kept at about  $80^{\circ}\text{C}$ . it was weighed again. From these two weights the water content was calculated.

Some of the experiments undertaken were designed to determine the effect of different environmental conditions on the gaseous exchange of the cotyledon. The factors studied were: (1) temperature, (2) available water supply, (3) light. The effects of two temperatures,  $20^{\circ}$  and  $25^{\circ}\text{C}$ ., were studied. The level of water availability was changed by varying the amount of water added to the beads in the cup of the apparatus at the beginning of the experiment. When this investigation was begun the experiments were conducted in a chamber in

which the light conditions could be regulated; daylight was excluded and the cultures were illuminated by a 1,000-watt lamp suspended above the bath in which they were immersed. By measuring the light intensity at the level of the cultures and through the same depth of water it was found that they were exposed to an intensity of about 100 f.c. The experiments on the effect of light were done in these conditions; the cultures exposed to light being illuminated continuously. In the later stages of the work circumstances had changed and it was not possible to ensure a rigid control of the light.

With the exception of the control series, which were kept in the dark in the observations on the effect of light, all other experiments were performed in the light; the purpose of this was to ensure as rapid a development as possible. Evidence has been presented elsewhere (Brown, 1941) which shows that, although a high light intensity depresses growth, nevertheless some light is necessary, especially during the earlier phase of development. Unfortunately, however, the light conditions throughout the whole series of experiments were not uniform. The experiments with isolated cotyledons were carried out with continuous light intensity of 100 f.c., while the experiments on the entire seed and on single cotyledons to which the embryo was attached were made with the intermittent conditions of ordinary daylight.

#### EXPERIMENTAL RESULTS

The experiments were designed to determine the relationship between the cotyledon and the rest of the seed. For this purpose three sets of observations involving gaseous exchange were made: (1) on the isolated cotyledon; (2) on the entire seed; (3) on the single cotyledon to which an embryo was still attached.

Since the emphasis is on the reactions of the cotyledon, a detailed study of these is required. This was undertaken by an examination of the effects of the following treatments on isolated cotyledons: (a) removal of the pellicle; (b) provision of different levels of water availability in the cultures; (c) exposure to light; (d) exposure to different temperatures.

The effect of pellicle removal from the entire seed was also examined, chiefly because the influence of the seed-coat on the gaseous exchange had already been examined by a number of earlier investigators.

##### *i. The gaseous exchange of isolated cotyledons.*

The effects of pellicle removal and of different levels of water availability are shown by the figures of Table I and by the curves of Fig. 2. The temperature in these experiments was 25° C. and the light conditions were continuous exposure at 100 f.c. The three sets of curves of Fig. 2 are each typical of the effect of a definite level of water availability.

The effects of pellicle removal at various levels of water availability are also shown by the data of Table II and by Fig. 3. The temperature in these experiments, however, was 20° C. The differences between the comparable



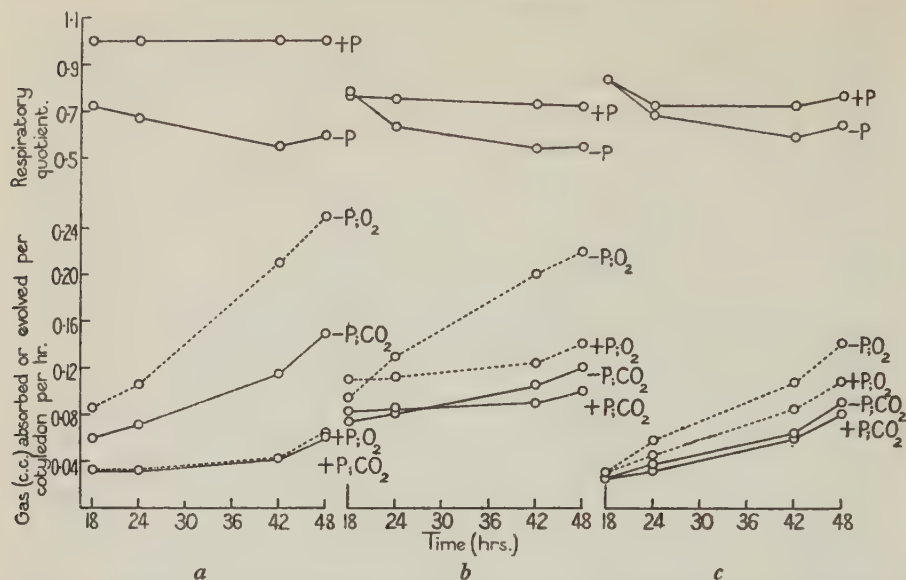


FIG. 2 (a-c). Effect of high (2a), intermediate (2b) and low (2c) levels of water availability on gaseous exchange of isolated cotyledons, with pellicle (+P) and without pellicle (-P). Temperature 25° C. Values plotted are given in Table I, expt. III.

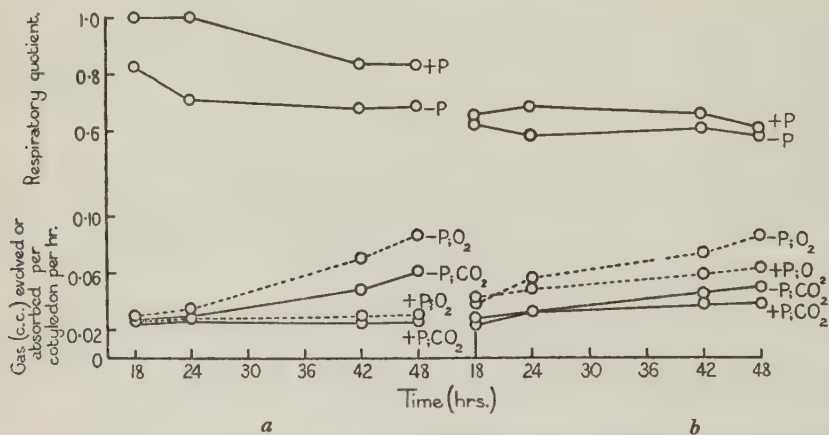


FIG. 3 (a and b). Effect of high (3a) and low (3b) levels of water availability on gaseous exchange of isolated cotyledons, with pellicle (+P) and without pellicle (-P). Temperature 20° C. Values plotted are those of Table II, expt. I.

data of Table II and Fig. 3, and those of Table I and Fig. 2, provide, therefore, an indication of the effect of temperature. The light conditions were the same in these and in the previous set of experiments.

The data of Tables I and II and of Figs. 2 and 3 indicate the following.

- (1) The rates of oxygen uptake and carbon dioxide production rise steadily from 18 to 48 hours.



TABLE I

*Gaseous Exchange of Isolated Cotyledons, with Pellicles (P+) and without Pellicles (P-), at Three Different Levels of Water Availability. The Data under I, II, and III in the Same Horizontal Series are from Replicated Experiments. Temperature 25° C. Rates as c.c. of O<sub>2</sub> absorbed or CO<sub>2</sub> evolved per Cotyledon per Hour. Water Content as Percentage of Fresh Weight.*

Time (hours)	I				II				III					
	CO <sub>2</sub> P+	P-	O <sub>2</sub> P+	P-	CO <sub>2</sub> P+	P-	O <sub>2</sub> P+	P-	CO <sub>2</sub> P+	P-	O <sub>2</sub> P+	P-	CO <sub>2</sub> /O <sub>2</sub> P+ P-	
(a) High level of water availability														
18	.	.	0.010	0.017	0.016	0.025	0.63	0.68	0.021	0.049	0.021	0.062	1.0	0.79
24	.	.	0.031	0.055	0.040	0.085	0.78	0.65	0.026	0.052	0.026	0.071	1.0	0.73
42	.	.	0.032	0.087	0.045	0.130	0.71	0.67	0.045	0.085	0.045	0.130	1.0	0.65
48	.	.	0.035	0.130	0.040	0.240	0.88	0.54	0.043	0.111	0.043	0.161	1.0	0.69
Water content:														
P+ 70 per cent., P- 78 per cent.														
(b) Intermediate level of water availability														
18	.	.	0.075	0.076	0.105	0.085	0.71	0.89	0.060	0.075	0.082	0.080	0.73	0.94
24	.	.	0.072	0.080	0.106	0.092	0.68	0.87	0.061	0.077	0.084	0.099	0.73	0.77
42	.	.	0.080	0.090	0.110	0.121	0.73	0.74	0.070	0.081	0.100	0.150	0.70	0.54
48	.	.	0.091	0.099	0.120	0.171	0.75	0.57	0.089	0.101	0.130	0.180	0.69	0.56
Water content:														
P+ 56 per cent., P- 61 per cent.														
(c) Low level of water availability														
18	.	.	0.040	0.040	0.050	0.050	0.80	0.80	0.050	0.044	0.050	0.058	1.0	0.76
24	.	.	0.050	0.049	0.067	0.061	0.75	0.80	0.051	0.056	0.065	0.073	0.79	0.77
42	.	.	0.062	0.061	0.082	0.100	0.76	0.61	0.075	0.080	0.095	0.102	0.79	0.78
48	.	.	0.071	0.082	0.094	0.134	0.76	0.61	0.081	0.084	0.105	0.143	0.77	0.59
Water content:														
P+ 42 per cent., P- 44 per cent.														
P+ 45 per cent., P- 51 per cent.														
P+ 37 per cent., P- 41 per cent.														



- (2) In nearly all series the rate of oxygen uptake tends to increase more rapidly than that of carbon dioxide production. Thus as development proceeds the value of the respiratory quotient tends to fall.
- (3) The removal of the pellicle always increases carbon dioxide production and oxygen uptake, but relatively the greatest increase is in the rate of oxygen uptake. Thus the removal of the pellicle tends to decrease the value of the respiratory quotient.
- (4) At 25° C. there are differences in the final water contents of the cotyledons corresponding to differences in water availability; at 20° C., however, these differences are not so apparent.
- (5) At 25° C. differences in the level of water availability induce corresponding differences in the rate of oxygen absorption and carbon dioxide production, the highest rate being attained at the highest levels of water availability. This relationship only applies to cotyledons without pellicles; when pellicles are present the highest rates are attained by cotyledons with an intermediate level of water availability.
- (6) At 20° C. different levels of water availability do not result in different rates in the gaseous exchange. The reaction of the cotyledons with pellicles is similar at this temperature to that at 25° C. With these conditions the rate of oxygen uptake is both absolutely and relatively low, resulting in a high respiratory quotient.
- (7) The differences between comparable series at 20° and 25° C. are considerable. Both rates of the gaseous exchange are lower at 20° C. than at 25° C.

The effect of light on the gaseous exchange is shown by the data of Table III. These results are from four replicated experiments in which the cotyledons were placed with their inner surfaces in contact with the glass beads and the exposed outer surfaces were all covered with pellicles. Experiments were undertaken to determine the effect of surface exposed and of pellicle removed in the light reaction. There was apparently no interaction between those variables and light; the results obtained are therefore not given here. The state or orientation of the cotyledon apparently did not affect its reaction to light.

In Table III the light and the dark experiments of each pair were conducted at the same time and performed with the two cotyledons from the same seed.

The data of experiments III and IV in Table III are shown graphically in Fig. 4. These data are chosen because they typify the two groups into which all the results of the series can be divided. These, like the previous set of results, all show a progressive increase with time in the rates of oxygen absorption and carbon dioxide production, the greatest relative increase being in the rate of oxygen uptake. In addition, the data indicate the following: (1) that the rate of oxygen uptake is greatest in dark, (2) the rate of carbon dioxide

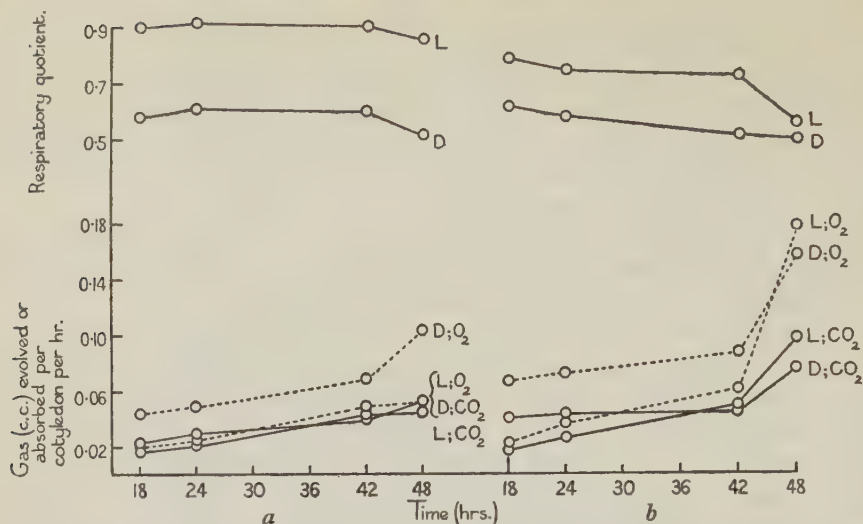


FIG. 4 (*a* and *b*). Effect on gaseous exchange of isolated cotyledons of exposure to light. L, exposed to light; D, light excluded; *a*, and *b*, are representative of two different types of results. Values plotted are given in Table III, 4, *a*, expt. III; 4, *b*, expt. IV.

TABLE III

*Gaseous Exchange of Isolated Cotyledons in Dark and Light. I, II, III, and IV represent Replicate Experiments. Rates as c.c. of O<sub>2</sub> absorbed or CO<sub>2</sub> evolved per Cotyledon per Hour.*

	Time (hours)	Dark			Light		
		O <sub>2</sub>	CO <sub>2</sub>	CO <sub>2</sub> /O <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	CO <sub>2</sub> /O <sub>2</sub>
I . . .	18	0.040	0.025	0.63	0.010	0.010	1.0
	24	0.048	0.030	0.63	0.013	0.013	1.0
	42	0.075	0.048	0.64	0.048	0.043	0.90
	48	0.065	0.040	0.62	0.045	0.040	0.89
II . . .	18	0.040	0.023	0.58	0.015	0.015	1.0
	24	0.053	0.033	0.62	0.023	0.018	0.78
	42	0.100	0.050	0.50	0.120	0.065	0.54
	48	0.140	0.070	0.50	0.115	0.060	0.52
III . . .	18	0.043	0.025	0.58	0.020	0.018	0.90
	24	0.049	0.030	0.62	0.025	0.023	0.92
	42	0.068	0.040	0.59	0.048	0.043	0.90
	48	0.103	0.053	0.52	0.053	0.045	0.85
IV . . .	18	0.065	0.040	0.62	0.023	0.018	0.78
	24	0.070	0.040	0.57	0.035	0.025	0.72
	42	0.085	0.043	0.51	0.060	0.045	0.75
	48	0.155	0.075	0.48	0.175	0.095	0.54

production is apparently scarcely affected by the light conditions, (3) that the depression in the rate of oxygen uptake in light does not always persist throughout the whole experimental period. In some—Fig. 4, *b*—the rate of oxygen uptake in the light suddenly increases after about forty-two hours.



ii. *The gaseous exchange of entire seeds.*

The rates involved in the gaseous exchange of entire seeds are shown in the data of Table IV, a typical set of results is also shown graphically in Fig. 5.

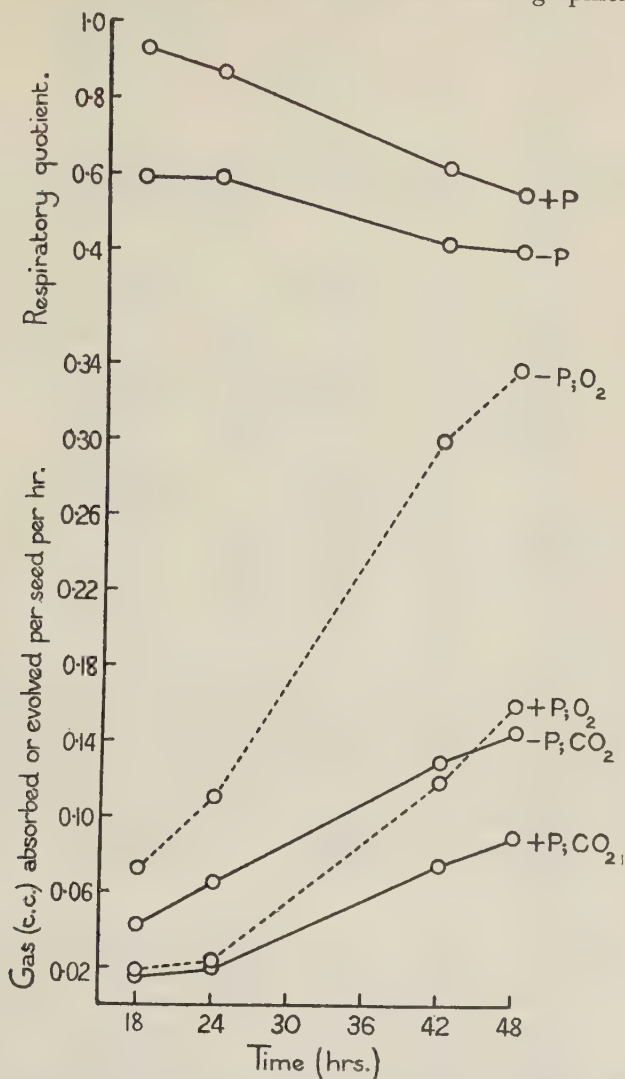


FIG. 5. Effect of pellicle on gaseous exchange of intact seed. +P with pellicle, -P without pellicle. Values plotted are given in Table IV, expt. III.

These data also show the effect of the removal of the pellicle from entire seeds. The seeds on which these observations were made were not from the same sample as those used for the cotyledon experiments; they were considerably smaller and probably older. The absolute values obtained cannot therefore be

TABLE IV

*Gaseous Exchange of Intact Seeds, with and without Pellicles. The data under I, II, III, and IV in the same Horizontal Series are from Replicate Experiments. Rates as c.c. of O<sub>2</sub> absorbed or CO<sub>2</sub> evolved per Seed per Hour.*

Time (hours)	I			II			III			IV		
	O <sub>2</sub>	CO <sub>2</sub>	CO <sub>2</sub> /O <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	CO <sub>2</sub> /O <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	CO <sub>2</sub> /O <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	CO <sub>2</sub> /O <sub>2</sub>
(a) With pellicles												
18	0.036	0.033	0.92	0.038	0.038	1.0	0.019	0.018	0.95	0.025	0.023	0.92
24	0.033	0.030	0.91	0.039	0.035	0.90	0.023	0.020	0.87	0.031	0.028	0.91
42	0.135	0.090	0.67	0.175	0.120	0.69	0.120	0.075	0.63	0.140	0.093	0.66
48	0.170	0.110	0.65	0.260	0.170	0.66	0.160	0.090	0.56	0.220	0.130	0.59
Water content: 42 per cent.				43 per cent.				45 per cent.				42 per cent.
(b) Without pellicles												
18	0.068	0.040	0.59	0.055	0.038	0.69	0.073	0.043	0.59	0.053	0.040	0.76
24	0.085	0.055	0.65	0.075	0.053	0.71	0.110	0.065	0.59	0.073	0.053	0.73
42	0.220	0.110	0.50	0.190	0.100	0.53	0.300	0.130	0.43	0.450	0.200	0.45
48	0.180	0.095	0.53	0.240	0.110	0.46	0.340	0.145	0.43	0.690	0.260	0.38
Water content: 52 per cent.				54 per cent.				59 per cent.				48 per cent.

compared directly with those given by the isolated cotyledons. In these experiments the temperature was 25° C. and an intermediate level of water availability was provided.

The course of the gaseous exchange of entire seeds is in general terms the same as that of isolated cotyledons. The rates of oxygen uptake and of carbon

TABLE V

*Gaseous Exchange of Cotyledons, with Embryo (E+) and without Embryo (E-). I and II are Duplicate Experiments. Rates as c.c. of O<sub>2</sub> absorbed or CO<sub>2</sub> evolved per Cotyledon per Hour.*

	Time (hours).	E+			E-		
		O <sub>2</sub>	CO <sub>2</sub>	CO <sub>2</sub> /O <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	CO <sub>2</sub> /O <sub>2</sub>
I . . . . .	18	0.025	0.020	0.80	0.020	0.018	0.90
	24	0.030	0.025	0.83	0.025	0.023	0.92
	42	0.130	0.085	0.65	0.045	0.033	0.73
	48	0.160	0.095	0.59	0.053	0.040	0.76
Water content: 45 per cent.		45 per cent.					
II . . . . .	18	0.078	0.050	0.64	0.033	0.028	0.85
	24	0.110	0.073	0.67	0.048	0.38	0.78
	42	0.250	0.140	0.56	0.073	0.058	0.80
	48	0.330	0.170	0.52	0.100	0.070	0.70
Water content: 53 per cent.		55 per cent.					

dioxide production continue to increase, the increase being greatest in the rate of oxygen uptake. Again, with entire seeds as with isolated cotyledons the removal of the pellicle occasions an increase of both rates; relatively greater in the case of oxygen uptake than in carbon dioxide production. A comparison of the quotients obtained with entire seeds and isolated cotyledons, however, reveals an important difference; with entire seeds the quotients tend to be lower.

iii. *The gaseous exchange of single cotyledons to which the embryo is attached.*

The data of Table V and Fig. 6 provide a comparison of the rates involved in the gaseous exchange of cotyledons attached to embryos and detached from them. The two sets of data (E + and E -) were obtained with the cotyledons from the same seed. The temperature in these experiments was 25° C. All the cotyledons were exposed to light and the pellicles were not removed.

In both series the changes with time in the rates of gaseous absorption and evolution are similar to those noted in other experiments. The difference between the two series are, however, pronounced. Both rates are markedly higher and the quotient lower when the embryo is present. The values of the quotient indicate that the increase is greatest in the rate of oxygen absorption.

## DISCUSSION OF RESULTS

The results reported indicate that the reactions of the cotyledons are profoundly affected by their degree of isolation from the rest of the seed. Many of the characteristics of the gaseous exchange of the isolated cotyledon, the

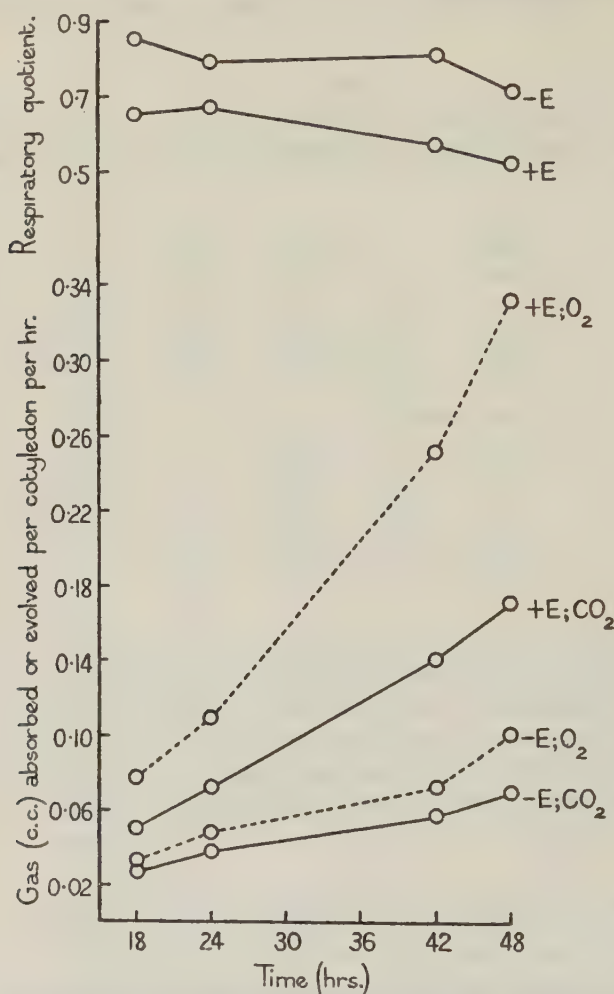


FIG. 6. Effect of embryo on gaseous exchange of cotyledon. +E embryo attached, -E embryo removed. Values plotted are given in Table V, expt. II.

entire seed, and the cotyledon to which an embryo is attached, show different similarities and dissimilarities in their gaseous exchange. The similarities are clearly the constant features of the internal factors of the material that determine the gaseous exchange; and the differences reflect corresponding variations in the operation of these factors. Thus the elucidation of the origins of the



differences involves the consideration of two separate problems: (1) the nature of the factors that determine the gaseous exchange, and (2) the nature of the internal conditions of the cotyledon that induce variations in the operation of these factors.

The absorption or evolution of gas must be due primarily to a process or set of processes involving a determinate series of chemical reactions, and for the elucidation of these processes two sets of data are of immediate importance: (a) the chemical changes that occur in the course of development, and (b) the rates of absorption and evolution of the reactant gases. Unfortunately, the data available do not represent the rates of exchange at the seat of the reaction but only at the surface of the tissue in which the reaction is occurring. Thus the consideration of these in relation to the larger problem of the nature and rates of the primary chemical reactions involves the consideration of two further matters: (a) the paths through the tissue along which gaseous diffusion occurs, and (b) the resistances established along those paths.

The arrangement of the discussion that follows is based on the above considerations, and the experimental results are, therefore, treated under the following headings:

- I. The physical conditions of the tissue that affect the exchange.
- II. The areas over which the exchange occurs.
- III. The chemical processes involved in the exchange.
- IV. The internal conditions that affect the exchange.
- V. The significance of the differences in the rates of the exchange that are related to the degree of isolation of the cotyledon from the rest of the seed.

I. The resistance of the diffusion path must, of course, affect the absolute rates, but since the properties of the gases are different it must also modify the relative rates. Thus both absolute and relative rates must be considered, and along with the absolute rates of absorption of oxygen and evolution of carbon dioxide, the ratio of these two quantities is of some importance.

Kostychev (1926) has discussed the factors which influence the respiratory quotient, enumerating the following: (1) the nature of the substrate, (2) the consumption of oxygen by processes other than respiration, and (3) the incidence of anaerobic along with normal aerobic respiration; anaerobic respiration being induced by the high resistance to oxygen diffusion of the tissues in the diffusion path. Wardlaw and Leonard (1936) have recently described a condition which would, if it developed, influence the quotient, but which is not covered by the factors enumerated by Kostychev. These workers showed that at a certain stage in the ripening of fruits an accumulation of carbon dioxide may occur in the tissues. Evidence is presented elsewhere (Brown, 1941) which shows that the carbon dioxide concentration in the intercellular system of the seed may reach 5 per cent. This, however, is a limiting value, which is not exceeded, however great the rate of respiration. Certainly there

is no reason to suppose this factor affects the value of the quotient after the first few hours. On the other hand, the data do not provide any evidence which definitely excludes this possibility; it is not impossible, for example, that the acidity of the cotyledon tissues changes in the course of development, with a consequent change in the quantity of carbon dioxide held in solution. The values of the quotients observed, however, can be satisfactorily explained without assuming any such complication, and evidence is presented below which shows that each of the factors enumerated by Kostychev is relevant to the discussion of the particular case considered here.

All the results presented here have one feature in common. Measurements of the gaseous exchange of the isolated cotyledon, of the single cotyledon to which an embryo is attached, and of the entire seed, all show that between 18 and 48 hours the rates of oxygen uptake and of carbon dioxide production increase and the values of the respiratory quotient decrease. The respiration of the entire seed of *Cucurbita Pepo* has also been examined in some detail by Stiles and Leach (1933), and their results, which cover the period of development studied in the present series of experiments, agree with those described in the last section. These workers found that the rate of carbon dioxide production continued to increase until about the fourth day, and that during the same period the value of the respiratory quotient continued to fall until it reached 0.6. During the first phase of germination the quotient increased from about 0.7 to about 0.95. These are features which are highly characteristic of the gaseous exchange of seeds, such as those of *Cucurbita*, in which the chief reserve material is oil. The oily seeds of apple (Harrington, 1923), of *Ricinus* (Murlin, 1933; Stiles and Leach, 1933), and of *Helianthus* (Stiles and Leach, 1933) all give trends similar to those of *Cucurbita*.

The high initial values obtained by Harrington (1923) and by Stiles and Leach (1933) with *Ricinus* and *Helianthus* were severally interpreted by these workers as due to the oxidation of reserve carbohydrates which had not been formed from fats. With *Cucurbita Pepo* the corresponding values, according to Stiles and Leach, cannot be due to this factor, since the carbohydrate content of dry seeds as determined by Peters (1861) and Laskovsky (1874) is not sufficiently great to sustain the observed rate of  $\text{CO}_2$  production. Clearly some factor other than the nature of the substrate is involved. The value of the quotient is higher than the oxidation of fats should yield under aerobic conditions, i.e. 0.7. Under anaerobic conditions, however, in which the consumption of oxygen is restricted, the quotient values observed might well arise. These are given by seeds and fragments of seeds, whether or not covered by the seed-coat membrane. Thus it would seem that if the high values given by the naked cotyledon are due to the development of anaerobic conditions, then the tissues of the cotyledon must have a low permeability to oxygen. Certain conclusions presented below suggest that such in fact is the case.

As development continues the consumption of oxygen increases at a greater

rate than does the corresponding evolution of carbon dioxide, which suggests that corresponding changes must occur in the permeability to oxygen of the cotyledon tissues. The anatomical changes that occur in the structure of the cotyledon, such as the formation of intercellular spaces, must promote the more rapid diffusion of gases, at least in the superficial layers of the cotyledon. Thus it is probable that at 48 hours the proportion of tissue respiring aerobically is substantially greater than it is at 18 hours.

With seeds and cotyledons to which pellicles are attached the high quotient values persist longer than with the naked structures. This would suggest that in this case there is a double restriction on the supply of oxygen to the developing tissue. All the results indicate that whatever the experimental material, whether whole seeds or isolated cotyledons, the effect of pellicle removal is always the same, i.e. an increase in the rate of  $\text{CO}_2$  production and a corresponding decrease in the value of the quotient. Similar effects consequent upon the removal of the seed-coat have been reported by Harrington (1923) with apple seeds and Frietinger (1927) with peas. These workers attributed the observed effects to the removal of the restrictive action of the seed-coat on the diffusion of oxygen. Stiles and Leach (1932) compared the respiration of the seeds of *Lathyrus* with and without their seed-coats. They found that with both there was a period of initial water uptake during which respiration increased rapidly; but whereas, when the seed-coats were not removed, this phase was followed by one of approximately constant respiration, when the seed-coats were removed this phase was not apparent. This difference they attributed to the restricting action of the testa on gaseous diffusion. The phase of constant respiration rate when the testa was attached was followed by a second period characterized by a rapidly increasing respiration rate, induced, these workers stated, by the breaking of the seed-coat. Stålfelt (1926) examined the interaction between the presence and absence of the seed-coat and the partial pressure of oxygen in the atmosphere in which the seeds were respiring. In air the removal of the seed-coat increased the respiration rate, but with oxygen concentrations greater than that of air the difference between seeds with and without their seed-coats tended to disappear. These results were taken to mean that the restricting effect of the seed coat is removed by the greater diffusion rate of oxygen through the membrane along the steeper concentration gradient in a higher percentage of oxygen. The properties of the pellicle of *Cucurbita* in relation to its permeability to gases have been described elsewhere (Brown, 1940). It was shown that carbon dioxide diffuses through this membrane four times as rapidly as oxygen, and that the volume of the latter that can diffuse into the seed from air is less than the average requirement of the cotyledon. Thus it is probable that in this case also the effects of the removal of the pellicle are due to the resulting enhanced oxygen supply to the respiring tissues.

It is probable, however, that the properties of the pellicle change with time. Even when the pellicle is attached to the seed, the consumption of oxygen increases at a greater rate than does the corresponding evolution of carbon



dioxide, which suggests that the membrane must be more permeable to oxygen in the later than it is in the earlier stages. It is probable that this change is due to the fact (Brown, 1940) that after prolonged contact with water the permeability of the seed-coat to gases increases. This effect, it was suggested, was related to the removal in solution of certain impermeable constituents of the membrane.

The presence of the pellicle modifies considerably the reaction of the cotyledon to the level of water availability. Whereas, when pellicles are absent, the highest rate of carbon dioxide production is attained with the highest level; when the pellicle is present, it is attained at 25° C. with the intermediate, and at 20° C. with the low levels of water availability (Figs. 2 and 3). Moreover the gaseous exchange at the high levels of water availability is characterized by a high quotient. A high quotient is also found in the earliest stages of germination, and in the discussion of this topic it was suggested that this indicated the presence of anaerobic respiration. The same considerations are relevant to the present data and they suggest the same inference. Certain other observations on the effect of water content on the permeability of the pellicle to gases are consistent with this view. In an earlier paper it was shown (Brown, 1940) that the saturated membrane is less permeable to gases than the unsaturated. It is probable that the saturated condition is induced by the highest levels of water availability with a consequent depression in the rate of oxygen diffusion to the respiring tissue.

II. The course of the gaseous exchange, its characteristics, and the effect upon it of certain experimental treatments are essentially similar in entire seeds and in isolated cotyledons. The similarity is particularly striking in view of the different circumstances of the two structures. The effects of pellicle removal are of particular interest in this connexion. When the pellicle is attached to the entire seed it envelops it completely, and all parts of the seed are then separated from the environment by this membrane. When the pellicle is attached to the isolated cotyledon it covers only the outer surface; the inner surface which in the seed is pressed against the other member of the pair of cotyledons is in the isolated cotyledon in immediate contact with the environment. In the isolated cotyledon the inner surface is free to absorb oxygen and so compensate for the restricted supply which reaches the outer surface when it is covered by the pellicle. Clearly no such compensating absorption occurs since if it did the removal of the pellicle should have no effect on the rate of oxygen uptake. This suggests that no oxygen reaches the outer surface by absorption through the inner, because it cannot traverse the intervening tissues, the permeability of these to oxygen being presumably very low. This conclusion carries two implications: (1) that respiration in the mass of the cotyledon when it occurs as the result of water uptake must be at least in part anaerobic, and (2) that the gaseous exchange over the two surfaces of the cotyledon must be independent. The significance of the first of these implications has been discussed in another connexion; the second suggests that, if the



gaseous exchanges over the two surfaces are independent, then it is possible that they are also different. The possibility was examined by comparing the respiration of a naked cotyledon with another from the same seed in which the pellicle had been transferred from the outer to the inner surface. The results of these experiments are shown in Table VI. One set of results is presented in a graphical form in Fig. 7. The results of this experiment are unfortunately not conclusive. They suggest the possibility, however, that the rate of oxygen absorption is higher over the outer surface than it is over the inner.

TABLE IV

*Gaseous Exchange of Cotyledons with Inner Surface blocked (B) and Inner Surface free (F). I, II, and III are Replicate Experiments. Rates as c.c. of gas absorbed or evolved per Cotyledon per Hour.*

	Time (hours)	B			F		
		O <sub>2</sub>	CO <sub>2</sub>	CO <sub>2</sub> /O <sub>2</sub>	O <sub>2</sub>	CO <sub>2</sub>	CO <sub>2</sub> /O <sub>2</sub>
I . . .	18	0.080	0.080	1.0	0.085	0.065	0.76
	24	0.110	0.081	0.75	0.105	0.070	0.67
	42	0.180	0.095	0.53	0.195	0.120	0.62
	48	0.205	0.100	0.49	0.235	0.150	0.64
II . . .	18	0.085	0.075	0.72	0.095	0.070	0.73
	24	0.095	0.080	0.84	0.105	0.075	0.71
	42	0.160	0.096	0.60	0.175	0.116	0.67
	48	0.195	0.112	0.58	0.220	0.150	0.68
III . . .	18	0.084	0.059	0.70	0.087	0.062	0.71
	24	0.086	0.060	0.70	0.118	0.071	0.60
	42	0.136	0.082	0.59	0.146	0.097	0.66
	48	0.194	0.106	0.55	0.206	0.138	0.67

III. Information is available on the chemical changes that occur in the germination of oily seeds, and much of this work has provided the basis for the interpretation of the characteristics of the gaseous exchange. Oil being the chief reserve food material it might be expected that this would be the ultimate source of the respiratory substrate, in which case the oxidation of the material either directly or after conversion to a carbohydrate form should yield a respiratory quotient of about 0.7. In fact in the later stages of development the quotient is much lower. The difference has been related by Harrington (1923), Murlin (1933), and Stiles and Leach (1933) to the fact that during the germination of oily seeds the percentage content of carbohydrate increases at the expense of the reserve oil. This has been demonstrated by Sachs (1859), Peters (1859), and Laskovsky (1875) for *Cucurbita*, by Miller (1910) for *Helianthus*, and by Murlin (1933) for *Ricinus*. In the formation of carbohydrates from fats large quantities of oxygen are consumed, but since all the carbohydrate formed is not immediately oxidized a part of the oxygen absorbed is not involved in respiration. In other words, in addition to the oxygen consumed in respiration a quantity of the gas is also absorbed by processes which do not involve a production of carbon dioxide, and this reduces the

quotient below the value characteristic of fat oxidation. So long as gaseous diffusion rate is not the controlling factor, the lower the quotient the greater is the rate of carbohydrate formation relative to its oxidation in respiration.

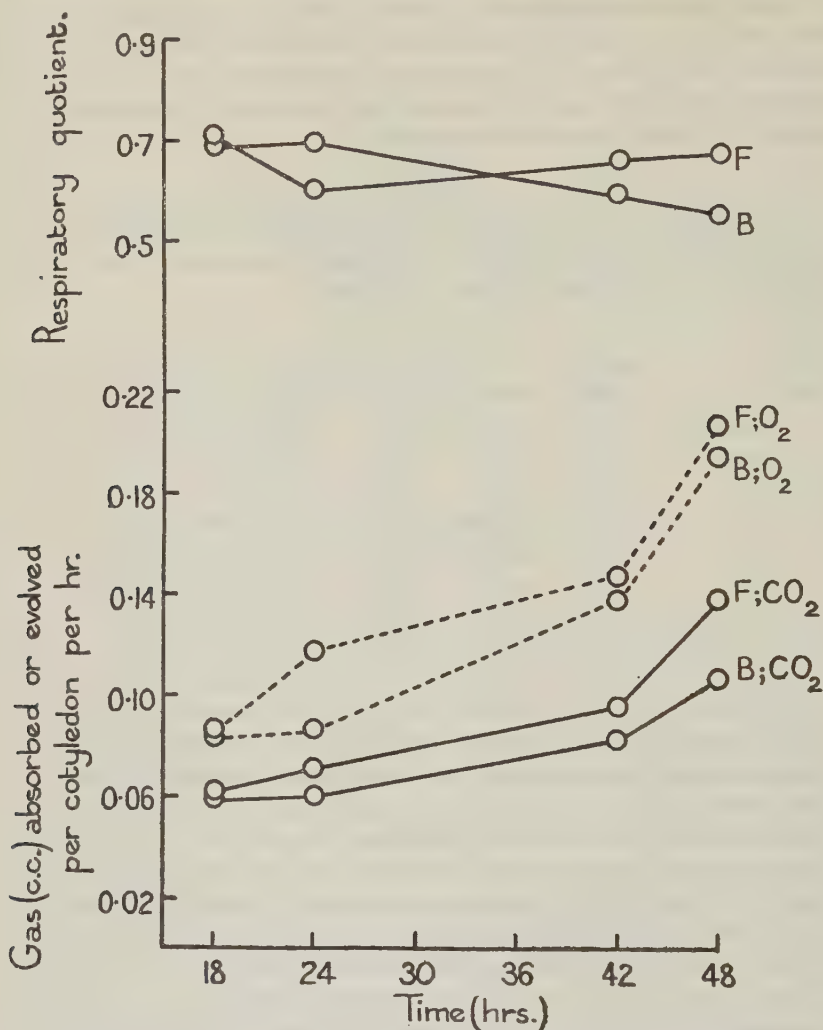


FIG. 7. Effect on gaseous exchange of blocking inner surface of isolated cotyledons. B, inner surface blocked; F, inner surface free. Values plotted are those of Table VI, expt. III.

The results obtained on the effect of various external conditions on the gaseous exchange indicate that these conditions affect not only the absolute but also the relative rates of the two processes concerned. Thus, the removal of the pellicle, a higher temperature, low light, and a high level of water availability all increase both the rates of exchange and decrease the value of the quotient. The interpretation of low quotient values presented above would

suggest that these treatments all increase the rate of respiration and of carbohydrate formation but that the relative increase is greater in the latter.

It is possible that the higher quotient obtained when the pellicle is present is the result not of a lower rate of carbohydrate formation but of the incidence of anaerobic respiration, induced by the low permeability of the pellicle for oxygen. But the increase in carbon dioxide production occasioned by the removal of the pellicle is frequently as low as 25 per cent. and, since the amount of carbon dioxide produced under aerobic conditions is very much greater than it is under anaerobic conditions for the same quantity of sugar consumed, the observed increase would represent a very small proportion of anaerobic respiration. Thus it is probable that the presence of the pellicle depresses the rate of oxygen uptake without inducing anaerobiosis, and the observed depression in the value of the quotient after the removal of the pellicle is therefore probably a reliable indication of an enhanced rate of carbohydrate formation.

The effect of a higher temperature is probably twofold, a direct effect on the chemical processes involved, and in part a more rapid diffusion of oxygen, to which the observed lower quotient is attributable.

The level of water availability apparently affects the rates of the gaseous exchange through its influence on the water content. When the pellicle is absent at 25° C., variations of this factor induce differences in the rates of the gaseous exchange with corresponding differences in water content; at 20° C. there is less effect on the gaseous exchange, with negligible effect on the water content. Clearly there is an intimate relationship between water content and the gaseous exchange. The nature of this relationship is, however, more clearly shown by a comparison of these results with those given by cotyledons in which the pellicle is still attached. At all levels of water availability the removal of the pellicle enhances the rates of the gaseous exchange without effect on the water content, which suggests that the metabolic activities that depend on the oxygen supply do not themselves, at least in the early stages, influence the water content, this being determined entirely by the colloidal properties of the cotyledon tissues. This conclusion suggests in turn that the correspondence between the water content and the gaseous exchange when the oxygen is unrestricted is due to the dependence on the water supply of the metabolic activities which demand oxygen. Thus the water content is a primary condition which is a determinant for the rates of gaseous exchange and therefore for carbohydrate accumulation and respiration.

At 25° C. differences in the level of water availability occasion different water contents; at 20° C. they do not. Clearly at 25° C. the level of water availability may be controlling while at 20° C. temperature is a controlling factor. At the lower temperature it is probable that the imbibitional pressure of the colloidal tissues of the cotyledon is not sufficiently great to promote a rate of absorption greater than that at which water is delivered at the surface of the beads, even at the lowest level of water availability.

The nature of the light effect is obscure. There is some indication that light has little if any effect on the rate of  $\text{CO}_2$  production, which if true suggests that the light effect is restricted to the process of carbohydrate formation.

IV. It is a fact of some interest that the treatments causing enhanced rates of respiration and carbohydrate accumulation also promote the most rapid development of the cotyledon (Brown, 1941). There is evidently an intimate relationship between these two processes and development.

In the growth of the seedling and also in the development of the cotyledon, processes of a fundamental character are involved, such as the elaboration of proteins and the formation of new cell-wall material which depend on a supply of carbohydrates. The higher the absolute rate of accumulation of carbohydrates, the higher, therefore, is likely to be the rate of development.

In germination there is a transition from a dormant to an active vegetative state. The process is characterized by certain anatomical changes in the developing tissues which on examination show that the process is gradual and does not occur uniformly throughout the cotyledon. The mass of active cells increases with time; simultaneously there is an increase in the respiration rate which is related to their increasing energy requirement. This interpretation corresponds with one developed by Kostychev (1926) based on the similarity between the curves for growth and respiration during germination; in this connexion he says: 'Die für architektonische Wachstumsvorgänge notwendige mechanische Energie entwickelt sich im Atmungsvorgänge; eine Zunahme des Energieverbrauches bedingt also eine entsprechende Steigerung der Energieproduktion.'

It is clear that certain experimental variables increase the rate of development by increasing the rates of respiration and of carbohydrate accumulation. The considerations put forward, however, do not exclude the reverse relationship that the gaseous exchange is itself affected by the development which it promotes.

V. Although the gaseous exchanges of the seed and of the cotyledon are similar, nevertheless they differ in detail and notably in relation to the final values of the respiratory quotient. These tend to be much lower with the entire seed than with the isolated cotyledon. This difference may be due either to the respiratory characteristics of the embryo itself or, on the other hand, may have its origin in an effect of the embryo on the metabolism and development of the cotyledon. Measurements of the gaseous exchange of the isolated embryo were made with the purpose of analysing this effect. The rates recorded are, however, of little interest because separation from the seed affects the growth of the embryo to a very much greater extent than that of the cotyledon. The values of the respiratory quotient are, on the other hand, of some significance. Throughout its development the respiratory quotient of the embryo never falls below 0.8; moreover, it is probable that the quotient is also that of the attached embryo. Schmidt (1891) claimed that fat could be transferred to the embryo in that form. Rhine (1926), however, presents



convincing evidence to the contrary, and it would seem that the embryo receives the cotyledonary food material in the form of carbohydrate. If such is the case, then the respiratory quotient of the attached embryo is likely to be high. Now, the quotient of the entire seed is lower than that of the isolated cotyledon, which suggests that in the entire seed the cotyledon must have a still lower quotient in order to compensate for the high quotient of the embryo. Thus there must be an effect of the embryo on the cotyledon which causes an enhanced development, inducing a higher absorption of oxygen.

Further evidence pointing to the same conclusion is provided by a comparison of the rates given by a cotyledon with and a cotyledon without an embryo, but from the same seed. The relevant data are those of Table V and Fig. 6. The presence of the embryo increases both rates but decreases the value of the quotient. When the embryo is attached, the volume of oxygen absorbed per hour is more than three times, and that of carbon dioxide produced more than twice, that of the isolated cotyledon. At this stage the embryo is minute and it is inconceivable that the very large increase could be due to the respiration of the embryo itself. That it is not so due is shown by the experiments of Fig. 8 in which the effect on the gaseous exchange of amputating the root, which forms the largest part of the embryo, was observed. If the difference between the cotyledons with and without an embryo is due to the respiration of the embryo then the removal of the root should, when the embryo is attached, have an immediate effect on the respiration of the whole. The experimental results show that the effect is negligible, and it may therefore be concluded that the enhanced gaseous exchange when the embryo is present indicates an effect of the embryo inducing a higher activity in the cotyledon.

#### SUMMARY

1. An apparatus is described in which a single cotyledon can be cultured, and by means of which the gaseous exchange of the single cotyledon can be measured.

2. Measurements were made of the rates of oxygen uptake and of carbon dioxide production of the entire seed, the isolated cotyledon, and the single cotyledon to which an embryo was attached. With each of these the effects of different temperatures, of light, of the presence and absence of the pellicle, and of different levels of water availability were studied.

3. Between 18 and 48 hours the rates of oxygen uptake and of carbon dioxide production continue to increase, and the value of the respiratory quotient to decrease from an initial high value to one of about 0.5. The high value is taken to indicate the existence of anaerobic respiration, and the final low values the accumulation of new respiratory carbohydrate. It is suggested that high rates of carbohydrate accumulation and of respiration indicate a high rate of development.

4. The removal of the pellicle increases both the rates of gas exchange and also increases the value of the quotient. These effects are related to the restricting action of the pellicle on the diffusion of oxygen to the respiring tissues.

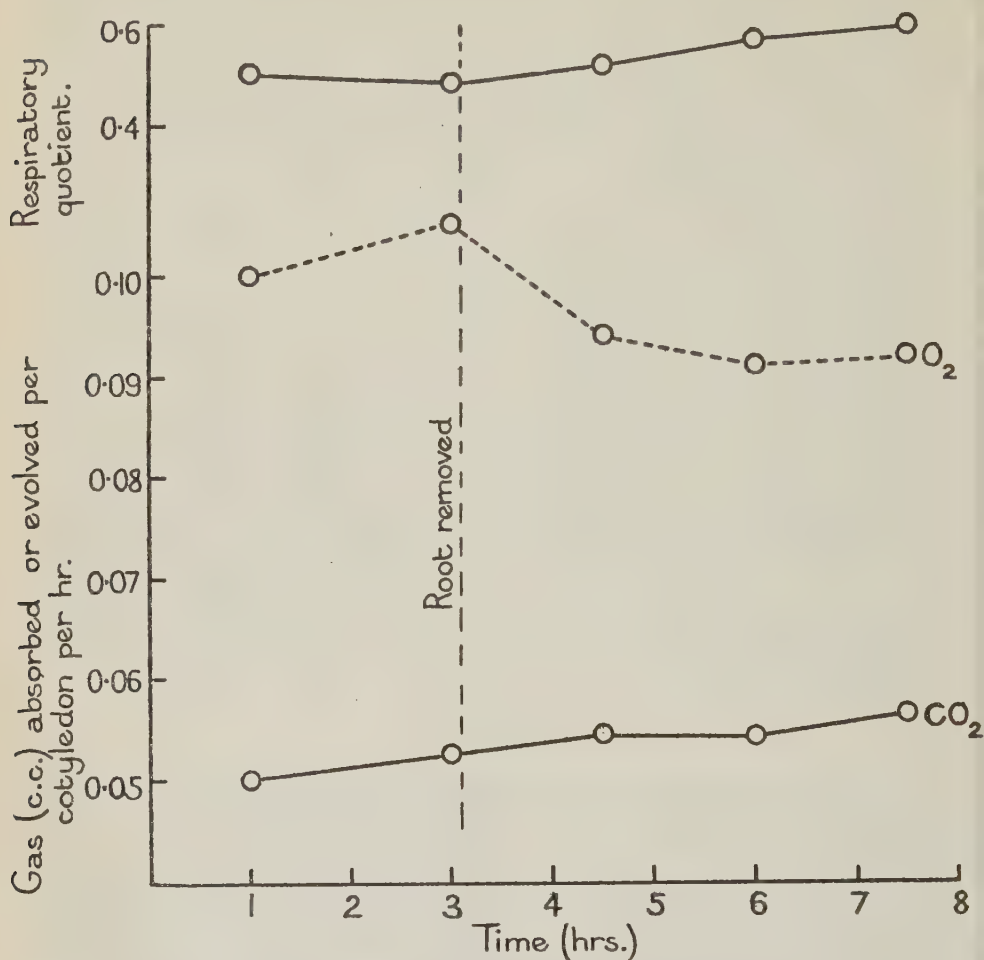


FIG. 8. Effect on the gaseous exchange of a cotyledon of amputating the root of the embryo attached to the cotyledon.

5. An increase in temperature occasions an increase in both the gas exchanges. The mechanism of this effect is discussed.

6. An increase in the level of water availability increases the rates of gas exchange and decreases the value of the quotient. These effects, it is shown, are due to the influence of this factor on the water content.

7. Light depresses the value of the quotient without having any consider-

able effect on the rate of carbon dioxide production. This is taken to indicate an effect of light on the mobilization of fats.

8. It is suggested that in the mass of the cotyledon respiration is partially anaerobic in the early stages of development.

9. When the embryo is attached to the cotyledon the rates of the exchange are higher and the value of the quotient lower than in its absence. This apparently indicates a stimulating effect of the embryo on the development of the cotyledon.

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# An Ecological and Taxonomic Study of the Algae of British Soils

## I. The Distribution of the Surface-Growing Algae<sup>1</sup>

BY

R. P. JOHN

### I. INTRODUCTION

CONSIDERABLE attention has been paid to the study of soil algae during the present century. Petersen (1935) gives a critical consideration of the literature to date, while Fritsch (1936) discusses the problems from a somewhat different angle. Both authors independently reach the conclusion that the subterranean (i.e. more or less deeply buried) algal flora, to which most attention has been given in recent years, is of little importance in soil-economy, since there is at present no evidence that multiplication is possible to any appreciable extent below the superficial layer of the soil. The presence of algae at a depth of several inches or even feet in the soil is attributed to the washing down of surface-forms by rain, aided by other agencies (Petersen, 1935, p. 85). Only those living at the surface can add to the supply of organic substance by photosynthetic activity, while certain Nostocaceae probably contribute to the fertility of the soil by nitrogen-fixation (De, 1939).

Few investigations have dealt with the algae present in the actual surface layers (Petersen, 1915, 1928*a*, 1932*a*, 1935). In Britain Bristol (1920, 1927) and James (1935) have examined the growth found at various depths in certain soils. In the present investigation the actual surface growth in a wide range of uncultivated soils has been studied in the hope of obtaining some insight into the ecology of soil-inhabiting algae. In many such soils the algal population is both varied and rich and includes a number of distinctive forms that have hitherto been rarely recorded.

Particular attention has been paid to the influence of the soil-reaction, which has already been investigated to some extent by Puymaly (1924) and Petersen (1928*b*). The relatively few chalk soils studied by me have a characteristic algal flora and a provisional list of possible calcicolous algae is given.

Since several of the characteristic subterranean algae have seldom or never been found on the actual soil-surface, Fritsch (1936, p. 201) suggests that the

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normal habitat of such forms may be just below the surface where a certain amount of light still penetrates. He postulates a microstratification of the growth in the surface-layers and suggests that the region occupied by active algae may extend to a depth of a few centimetres.

## II. THE SOILS AND THEIR MODE OF COLLECTION AND CULTURE

(a) *Mode of collection.* Only the surface soil was collected. Samples from the top, the second, the third, and the fourth centimetre respectively were gathered once a month for nearly two years from Epping (21),<sup>1</sup> Oxshott Heath (22), and Box Hill (7) in order to study the microstratification. These samples were collected with the help of a small rectangular shovel, the edges being 1 cm. high, while the front was produced into a triangular blade with sharp cutting-edges. Selecting a spot with few or no stones, two holes were dug about 8 in. apart, and a small amount of the soil from the adjacent edges of the holes pared off with a sterile scalpel so as to expose a clean surface. The shovel was then inserted at one hole, at a depth of 1 cm. below the surface, and pushed through to the other. Any loose soil left on the surface was scraped off before the second layer was collected. At Box Hill grass roots and the many flints made collection by this method difficult. In wet weather a block of the moist soil could be removed and slices cut off with a sharp knife. When the soil was dry it was scraped off the surface, approximately to a depth of 1 cm.; then the loose soil was removed and the exposed surface sterilized with a hot scalpel before a further lot of soil was removed for the second centimetre.

All the samples, except those just named, were collected only on one or two occasions. The soil down to a depth of about 4 cm. was gathered with a small trowel, thoroughly mixed, and placed in a waxed tin until cultures were started. Five air-dried samples from the grass-heath of Breckland, collected to a depth of 15 cm. in 1936 by Dr. A. S. Watt and stored in bottles for nearly three years (Watt, 1940), were also placed at my disposal.

The pH value of the soils was determined by the colorimetric method using a B.D.H. capillator; that of the five Breckland soils had been determined by the hydrogen electrode method. The percentage of  $\text{CaCO}_3$  was estimated with a Collin's calcimeter. Of the 24 soils studied, 15 were alkaline, 7 acid, and 2 neutral. The pH values varied between 8.4 and 3.7, so that the range was much wider than that available to Petersen (1928b). Mechanical analyses (see Table IV) of the soils from Epping, Oxshott, and Box Hill were made according to the Revised Official Method of Mechanical Analyses ('Journ. Agric. Sci.', xviii. 734-9, 1928).

(b) *Methods of culture.* Half-pint bottles, filled to a depth of 2 in. with Benecke solution (Kufferath, 1930, p. 46) and closed with a plug of cotton-wool covered by brown paper tied over the mouth of the bottle, were sterilized in an autoclave at a pressure of 20 lb. for 20 minutes. After cooling, about

<sup>1</sup> The numbers relate to the list of soils on p. 325.

1 c.c. of soil was introduced into the bottle with a sterile spatula. Such cultures are referred to as *liquid cultures*. Others were prepared by spreading a layer of soil, about 1 cm. deep and moistened periodically with sterilized distilled water, in a Petri dish covered with a sheet of glass, both previously sterilized. These cultures are referred to as *moist cultures*. This method, in which the soil is kept under approximately natural conditions, was mostly very successful. Both types of cultures were placed in an unheated window-box. During summer the liquid ones had to be kept in semi-darkness for a day or two before examination, since otherwise the large amount of reserve food in the algal cells, mostly in the form of fat, rendered an elucidation of the cell-structure impossible.

In making sub-cultures a few cells were drawn into a fine pipette, washed in sterile distilled water, and transferred to 1½ per cent. Benecke agar in Petri dishes. When this was not feasible, a few drops of the culture were shaken up with sterile distilled water and poured on to the surface of an agar plate, the excess being drained off. Another method, useful for isolating unicellular forms and already used for bacteria, was to deposit a drop from the liquid culture at one side of an agar plate, after which the drop was drawn across the plate along 4 or 5 lines with a platinum wire, followed by lines perpendicular to these, and so on. After a week or two individual growths could be picked up by means of a pipette and transferred to fresh agar plates, although in some instances better growth was obtained by transference to liquid cultures. No bacteria-free cultures were attempted.

(c) *The soils and their algal growth.*

(1) Sand-dunes near Llandudno, N. Wales; firm sand with a certain amount of humus; July; pH 8·4; CaCO<sub>3</sub> 2·15 per cent.: *Chroococcus minutus*, *Tolypothrix tenuis* f. *terrestris*, *Anabaena variabilis*, *Crinalium magnum*, *Achnanthes coarctata*, *Carteria arenicola*, *Muriella magna*, *Hormidium nitens*, *Pleurastrum terrestre*, *Heterothrix exilis*.

(2) Whipsnade, Herts., hill-side with short grass; chalky clay with flints; June; pH 8·2; CaCO<sub>3</sub> 77 per cent.: *Tolypothrix tenuis* f. *terrestris*, *Nostoc commune*, *Phormidium Hieronymusii*, *Chlamydomonas* sp., *Chlorococcum lobatum*, diverse Palmellaceae, *Hormidium nitens*, *Pleurastrum terrestre*, *Heterothrix exilis*.

(3) Gog Magog Hills, Cambridgeshire; pure chalk from quarry; November; pH 8·2; CaCO<sub>3</sub> 90·3 per cent.: *Phormidium foveolarum*, *P. Hieronymusii*, *Microcoleus vaginatus*, *Navicula atomus*, *Chlamydomonas calcicola*, *C. sp.*, *Hormidium nitens*, *H. crenulatum* var. *a*, *Cylindrocystis Brebissonii*, Chrysophyceae III.

(4) Gaydon, Wales, mixed oak and ash wood with little undergrowth; soil chalky; July; pH 8·2; CaCO<sub>3</sub> 14·6 per cent.: *Chroococcus minutus*, *Tolypothrix tenuis* f., *Nostoc* sp., *Phormidium foveolarum*, *Nitzschia tryblionella* var. *debilis*, *Chlamydomonas* sp., *Chlorococcum* sp., *Chlorochytrium paradoxum*, *Hormidium nitens*, *Pleurastrum terrestre*, *Polyedriella helvetica*.

(5) Breckland, grass heath (Watt, A); highly calcareous, shallow soil; November; pH 8·2; CaCO<sub>3</sub> 17 per cent.: for list of algae, see Table III.

(6) Sloping grassland near Loch Doon, Kirkcudbright; July; pH 8·2; CaCO<sub>3</sub> 0·3 per cent.: *Chroococcus minutus*, *Cylindrospermum alatosporum*, *Anabaena torulosa*,



*Eunotia tenella*, *Achnanthes linearis*, *Pinnularia viridis*, *Hantzschia amphioxys*, *Nitzschia palea*, *Chlamydomonas gloeocystiformis*, *C. platyrhyncha*, two members of Palmellaceae, *Chlorococcum* sp., *Chlorella vulgaris*, *Muriella magna*, *Hormidium nitens*, *Cosmarium holmiense* var. *integrum*, *Pleurochloris* sp.

(7) Box Hill, Surrey, slope with short grass; chalky clay with flints; December 1937 to July 1939; pH 8.0;  $\text{CaCO}_3$  51.3 per cent.: *Nostoc commune*, *N. paludosum*, *Phormidium foveolarum*, *P. Hieronymusii*, *P. autumnale*, *Microcoleus vaginatus*, *Navicula atomus*, *N. dicephala*, *Nitzschia palea*, *Chlamydomonas calcicola*, *C. sp.*, *Chlorococcum lobatum*, *Chlorochytrium paradoxum*, *Coccomyxa dispar*, *Hormidium nitens*, *Stichococcus bacillaris*, *Pleurastrum terrestre*, *Vaucheria sessilis*, *Botrydiopsis anglica*, *Pleurochloris* sp., *Bumilleriopsis Peterseniana*, *Heterothrix exilis*.

(8) Loch Ken, Kirkcudbright, beech wood on a hill-side; July; pH 8.0;  $\text{CaCO}_3$  nil: *Chlamydomonas* sp., *Chlorococcum* sp., *Coccomyxa dispar*, *Hormidium nitens*, *Stichococcus bacillaris*, *Euglena mutabilis*, *Heterothrix exilis*.

(9) Sand-dunes near Llandudno, N. Wales, close to No. (1); sand without humus; July; pH 8.0;  $\text{CaCO}_3$  4.0 per cent.: *Chlorococcus minutus*, *Nostoc commune*, *Crinalium magnum*, *Phormidium foveolarum*, *Achnanthes coarctata*, *Carteria arenicola*, *Hormidium nitens*, *Pleurastrum terrestre*, *Heterothrix exilis*, *Bumilleriopsis Peterseniana*.

(10) Oak wood, Fairy Glen, N. Wales; July; pH 8.0;  $\text{CaCO}_3$  1.2 per cent.: *Nostoc* sp., *Phormidium foveolarum*, *Hantzschia amphioxys*, *Hormidium nitens*, *Heterothrix exilis*.

(11) Bracken-covered hill-side facing sea, Bangor, N. Wales; soil very sandy; July; pH 8.0;  $\text{CaCO}_3$  nil: *Nostoc humifusum*, *Chlamydomonas pseudoelegans*, *C. minutissima*, *Polyedriella helvetica*.

(12) Wicken Fen, Cambridgeshire, sedge-area; soil peaty; September, November; pH 7.8;  $\text{CaCO}_3$  nil: *Phormidium autumnale*, *Diploneis elliptica*, *Amphora Normani*, *Nitzschia palea*, *Chlamydomonas* sp., *Pleurastrum terrestre*, *Oedogonium* sp., *Heterothrix exilis*, Chrysophyceae I.

(13) Oak wood, Maidstone, Kent; clay soil; April; pH 7.8;  $\text{CaCO}_3$  nil; *Tolypothrix tenuis* f. *terrestris*, *Nostoc* sp., *Oscillatoria formosa*, *Phormidium foveolarum*, *Schizothrix arenaria*, *Chlamydomonas elliptica* var. *britannica*, *Dactylococcus bicaudatus*.

(14) Breckland grass heath (Watt, B); rather dry, slightly calcareous soil; November; pH 7.8;  $\text{CaCO}_3$  1.6 per cent.: for list of algae, see Table III.

(15) Mixed wood with oak and ash, Skegness, Lincs.; soil clayey; June; pH 7.4;  $\text{CaCO}_3$  nil: *Tolypothrix tenuis* f. *terrestris*, *Cylindrospermum licheniforme*, *Achnanthes linearis*, *Pinnularia viridis*, *P. intermedia*, *Nitzschia palea*, *Chlamydomonas* sp., *Macrochloris dissecta*, *Chlorococcum* sp., *Hormidium nitens*, *Monodus* sp.

(16) Pine woods, Rowardennon, Loch Lomond, no undergrowth; soil sandy; June; pH 7.2;  $\text{CaCO}_3$  nil: *Chlamydomonas* sp., Palmellaceae, *Chlorococcum lobatum*, *Chlorella vulgaris*, *Muriella magna*, *Coccomyxa dispar*, *Hormidium nitens*, *Cosmarium cucurbita*, *Vischeria stellata*.

(17) Heath with *Erica* and *Ulex*, Bournemouth; October; pH 7.2;  $\text{CaCO}_3$  nil: *Hormidium nitens*, *Cosmarium cucurbita*.

(18) Oak wood, Rowardennon, Loch Lomond, with undergrowth of Bracken and grass; soil very sandy; June; pH 6.6;  $\text{CaCO}_3$  nil: *Eunotia tenella*, *Chlamydomonas* sp., *Chlorococcum lobatum*, *Dactylococcus bicaudatus*, *Muriella magna*, *Coccomyxa dispar*, *Hormidium nitens*, *Cosmarium cucurbita*, *Vischeria stellata*.

(19) Hill-Slope, Widcombe-in-the-Moor (Dartmoor); soil very peaty; January;



pH 6.6;  $\text{CaCO}_3$  nil: *Phormidium autumnale*, various Diatoms, *Chlamydomonas pseudogloeogama*, *Chlorococcum lobatum* var. *tenue*, *Hormidium nitens*, *Cylindrocystis Brebissonii*, *Cosmarium cucurbita*, Chrysophyceae I.

(20) Breckland grass heath (Watt, C), with continuous turf; November; pH 6.2;  $\text{CaCO}_3$  0.13 per cent.: for list of algae, see Table III.

(21) Mixed wood with beech, hornbeam, and birch, Epping Forest, no undergrowth; soil sandy with clay and silt; December 1937 to July 1939; pH 5.2;  $\text{CaCO}_3$  nil: *Chlamydomonas* spp., *Chlorococcum humicolum*, *Chlorella vulgaris*, *Muriella magna*, *Coccomyxa dispar*, *Hormidium nitens*, *Pleurastrum terrestre*, *Zygogonium ericetorum*, *Pleurochloris acidophila*, *Euglena mutabilis*.

(22) Open heath, Oxshott, Surrey; soil very sandy; December 1937 to July 1939; pH 4.6;  $\text{CaCO}_3$  nil: *Chlamydomonas subangulosa*, *C. sp.*, *Carteria acidicola*, *Chlorococcum lobatum* var. *tenue*, *Borodinella polytetras*, *Chlorella vulgaris*, *Muriella magna*, *Coccomyxa dispar*, *Hormidium nitens*, *Stichococcus bacillaris*, *Pleurastrum terrestre*, *Cosmarium cucurbita*, *Euglena mutabilis*, Chrysophyceae II, *Pleurochloris acidophila*.

(23) Breckland grass heath (Watt, D), with patches of *Cladonia sylvatica*; soil very deep; November; pH 4.4;  $\text{CaCO}_3$  nil: for list of algae, see Table III.

(24) Breckland grass heath (Watt, G), infertile area with *Cladonia sylvatica* and *Festuca ovina*; November; pH 3.7;  $\text{CaCO}_3$  nil: for list of algae, see Table III.

### III. GENERAL CONSIDERATION OF THE ALGAL GROWTH

#### (a) *The General Characteristics of the Flora.*

All the soils examined included algal germs capable of growth, even some collected from the stalactite caves in Kent's Cavern, Devonshire, containing at least three species. Most soils, however, bore no visible algal growth at the time of collection. In Epping Forest and on Oxshott Heath conspicuous growths of *Zygogonium ericetorum*, often mixed with a few filaments of *Hormidium nitens*, were found not far from the place of collection. The Gog Magog soil (3) also bore a light green growth of *H. nitens*. At Box Hill *Nostoc commune* was frequently found, and very occasionally *Phormidium foveolarum* and *P. autumnale*, together with *Microcoleus vaginatus*, formed strata on the soil surface, while *Vaucheria sessilis* and *V. terrestris* were conspicuous on the exposed chalk near at hand.

The smallest number of algae found in any sample was 8. The occurrence of numerous species in certain soils (40 from Box Hill, 34 from Loch Doon, cf. Table I), less than the full number since all the forms present could not be identified, is noteworthy, since it has been assumed that uncultivated soils contain fewer species than cultivated ones. The largest number of species found in the Broadbalk soils (Bristol Roach, 1927) was 35, while the average number in the 44 soils examined by Bristol (1920) was between 10 and 11, whereas in the soils here considered it is nearly 18. The discrepancy is no doubt partly due to our improved knowledge of the unicellular and colonial algae, but also probably to the preliminary drying of the soil. The

British soils examined by me are also richer in species than the Danish ones. Some of the Iceland soils studied by Petersen (1928a) contain more species, but these are largely Diatoms, whereas in the British soils the algal growth is composed chiefly of green forms. Nineteen of the soils examined showed a preponderance of green algae over Heterokontae, Diatoms, and Cyanophyceae.

Most of the common algae enumerated by Bristol (1920, 1927) occur also in the soils under discussion. The most noticeable difference is the absence of *Plectonema Battersii*, which would seem to be largely confined to cultivated soils, as James also failed to find it. Fritsch (1936, p. 196) has already pointed out that the *Ulothrix subtilissima* var. *variabilis* of Bristol is probably a species of Hormidium, threads of which are universally present. Several new species have been found to be important constituents of the soil flora, while a number of algae described by Petersen from Iceland and Denmark, but not hitherto recorded for Britain, have been encountered. Of the new species, *Pleurastrum terrestre* occurred in many of the samples and is possibly present in others, since it is difficult to recognize in the liquid cultures in which it exists in a unicellular state. *Muriella magna* appears to be another common British soil form. *Interfilum paradoxum*—a curious alga recorded by Chodat (1922) as an epiphyte on an infusorian—was found quite commonly in two acid soils, while another variety of the same species occurred in all the Breckland soils. Another interesting find was *Borodinella polytetas*, which has not been met with since it was first described by Miller (1927). *Pseudendoclonium basiliense* var. *Brandii* Vischer, found at Box Hill, constitutes the first record of the genus from the soil, though *P. submarinum* has been found on woodwork in Iceland by Petersen (1928a). *Macrochloris dissecta* and *Fernandinella alpina* var. *semiglobosa* are new records for Britain, though already recorded from the soil by Petersen.

Among Heterokontae *Bumilleriopsis Peterseniana*, *Heterothrix quadrata*, *Heterococcus caespitosus*, *Polyedriella helvetica*, and *Vischeria stellata* are new records for this country, while two new species of *Pleurochloris* are described (see Part II). Among Cyanophyceae *Crinalium magnum* is new, while *Phormidium Hieronymusii* has not been found in Britain before.

#### (b) *The relation to the pH of the soil.*

The considerable range in pH admits of a contrast between the growth on soils with an acid and those with an alkaline reaction. The number of species belonging to the various classes found in the different soils is shown in Table I, while the frequency of the commoner species in some of the soils is given in Table II.

Blue-green algae show a distinct preference for alkaline soils (Table I). The average number of species found in such soils is between 4 and 5, whereas in acid soils it is under 1. The average frequency (total number of times blue-green algae have occurred in the soils divided by the number of soils) is 4.0 and 0.5 respectively. Of the 20 species of Cyanophyceae recorded, 18 oc-

curred exclusively in the alkaline soils and only 2 (*Phormidium autumnale*, *Plectonema nostocorum*) in both acid and alkaline soils. *P. autumnale* was found in 9 out of the 15 alkaline soils, while it was present only in 2 acid soils, with a pH exceeding 6; Petersen (1928b, p. 11) also records it from Iceland soils, with a pH of above 6. *Plectonema nostocorum* occurred commonly in the

TABLE I

*Representation of the Different Classes of Algae on the Various Soils*

Soil No.	Nature.	pH.	Cyan.	Diats.	Heterok.	Chloro.	Chryso.	Rhodo.	Total.
1.	Sand-dune, rather firm sand	8.4	4	3	1	5	—	—	13
2.	Chalky grassland	8.2	5	4	2	8	—	—	19
3.	Chalk quarry	8.2	4	5	3	8	1	—	21
4.	Oak woods, chalky clay	8.2	5	3	3	7	—	—	18
5.	Chalky grass heath	8.2	7	3	6	7	—	1	24
6.	Grassland	8.0	6	8	1	19	—	—	34
7.	Grassland, chalk	8.0	6	8	5	20	1	—	40
8.	Beech wood	8.0	0	2	2	9	—	—	13
9.	Sand-dunes, loose sand	8.0	8	2	2	6	—	—	18
10.	Mixed wood with oak and ash	8.0	4	2	1	3	—	—	10
11.	Bracken, sandy soil	8.0	1	1	2	4	—	—	8
12.	Fenland	7.8	2	8	3	7	1	—	21
13.	Oak woods	7.8	9	3	2	7	1	—	22
14.	Grass heath, slightly chalky	7.8	3	1	7	9	1	1	22
15.	Mixed wood	7.6	3	9	2	7	—	—	21
Approximate averages for alkaline soils			4.5	4	3	8.9	—	—	20
16.	Pine wood	7.2	0	0	1	9	—	—	10
17.	Heath	7.2	0	0	1	8	—	—	9
18.	Oak wood	6.6	0	2	1	10	—	—	13
19.	Moorland, peaty soil	6.6	1	4	2	9	1	—	17
20.	Grass heath	6.2	2	1	5	14	1	1	24
21.	Beech wood	5.2	0	2	1	14	1	—	18
22.	Heath	4.6	0	1	1	18	1	—	21
23.	Grass heath	4.4	0	1	2	13	—	—	16
24.	Grass heath	3.7	0	0	1	12	—	—	13
Approximate averages for acid soils			1	1.2	2	13	—	—	18



alkaline Breckland soils A and B, and was found in soil 20 only once; this soil is peculiar in containing other species present in alkaline soils. It may be concluded that the two species just considered are typical of alkaline soils, although found occasionally also in those with a slight degree of acidity.

Diatoms are also more characteristic of alkaline than of acid soils, though the difference is less marked; the average number of species is 4 in the alkaline and between 1 and 2 in the acid soils. The average frequency (see above) in the alkaline soils is 4 and in the acid 1.6. Seventeen of the 24 species occurred only in alkaline soils, and no Diatom was found exclusively in acid soils. Petersen (1915, p. 282) records *Navicula falaisensis*, *Pinnularia subcapitata*, and *Stauroneis aerophila* as restricted to acid soils in Denmark, but none of these occurred in my samples. He lists 19 species for neutral and 9 species for acid soils, the average number per soil being about equal, and concludes (p. 354) that the chemical nature of the soil has little effect on the distribution of Diatoms. Neither Fritsch and Salisbury (1915) nor Petersen (1935) record Diatoms on moist heaths, but the latter suggests that other factors may be responsible for their absence, since species of *Eunotia* and *Pinnularia* were common on very acid moors. Richter (1906, pp. 35, 76) found that Diatoms do not grow in cultures on acid media.

*Eunotia tenella* was very common in soil 18 (pH 6.6), while it was found only once in the alkaline soils. *Pinnularia borealis*, and to a less extent *Navicula atomus*, are present in both types of soils. The latter species is found in the Breckland soils down to a pH of 4.36, but is absent from G with a pH of 3.7; its distribution seems also to be affected by the  $\text{CaCO}_3$  content (p. 334). It grows more abundantly in the more alkaline soils (Breckland A and Box Hill). The commonest soil Diatom is *Hantzschia amphioxys*, which was found in 13 of the 15 alkaline soils, although only in 2 of the 7 acid ones. *Nitzschia palea* is rather common in 6 of the alkaline soils, although never found in acid ones.

The Heterokontae likewise show a preference for alkaline soils, the average number of species being 3 in the alkaline and about 2 in the acid soils. The commonest is *Heterothrix exilis*, and this is much more frequent in the alkaline soils. *H. quadrata* and *Heterococcus Chodati* are confined to alkaline soils, while *H. caespitosus* occurred in Breckland soils down to a pH of 4.4, but grew best in the alkaline ones. *Bumilleriopsis Peterseniana*, though common only in the alkaline soils, was occasionally found in the acid Breckland soils C and D. Species of *Pleurochloris* occur in both types of soils, but *P. acidophila* has been met with only in acid ones, while *Polyedriella helvetica* is found only in alkaline soils (4, 11). *Vischeria* occurred only in a slightly acid and a neutral soil (16, 19). Petersen (1928b) records *Heterothrix exilis*, *Bumilleriopsis Peterseniana*, and *Heterococcus viridis* from acid soils, and most of these have also been found in the acid soils examined by me.

Unlike the classes previously considered, the Chlorophyceae are more abundant in acid than in alkaline soils (Table I). When all the species are



taken into consideration, the alkaline show an average of between 8 and 9, while the acid soils have an average of 13 species. While 13 species were rather common in the alkaline soils, 16 were common in the acid soils, the average frequency of the common species being between 2 and 3 in the alkaline and about 5 in the acid soils.

Of the Volvocales, *Carteria arenicola* was found only in the two alkaline sand-dune soils, while *C. acidicola* occurred only on the acid Oxshott Heath. This species grows best in a culture solution, with a pH of about 3. Most of the species of Chlamydomonas are confined to a single soil. *C. calcicola*, which is common in the alkaline soils (3, 5, 7, 14, 20), was also found in the acid Breckland soils. *C. subangulosa* was confined to acid heaths (22-4).

Many Chlorococcales appear to flourish equally well in both types of soils, although *Fernandinella alpina* var. *semiglobosa* and *Chlorochytrium paradoxum* were confined to alkaline soils, and *Borodinella polytetras*, *Trebouxia arboricola*, *Chlorella vulgaris*, and *Coccomyxa* sp. to acid soils. Petersen (1915) concludes that *Dactylococcus bicaudatus* and *Coccomyxa dispar* are characteristic of acid soils, but both grew equally well in both of the types of soil examined by me. *Muriella magna* is only common in acid soils.

The Ulotrichales are represented only by a limited number of species. *Hormidium nitens* has been found in all the soils examined. *H. crenulatum* var. *b* and *Interfilum paradoxum* occurred only in those with an acid reaction, while *Hormidium crenulatum* var. *a* was confined to alkaline soils. In spite of the considerable degree of resemblance between the three forms of Hormidium, this probably indicates a specific difference (Strøm, 1928). The Chaetophorales are represented only by *Pleurastrum terrestre* and *Pseudendoclonium basiliense* var. *Brandii*. The former was equally frequent in both types of soils, though that occurring in acid soils is probably a distinct variety. The latter was found with certainty only in the Box Hill soil. *Vaucheria terrestris* and *V. sessilis* were confined to the Box Hill soil (cf. Petersen, 1915, p. 360).

The Conjugales are represented mainly by Desmids and these are not widespread. *Closterium pusillum* var. *monolithum* occurred in the alkaline soil 6 and in the slightly acid soil 19, while *Cosmarium cucurbita* grew abundantly in a number of acid and neutral soils (pH 4.0-7.2). *Cylindrocystis Brebissonii*, though found both in alkaline and acid soils, was met with in abundance only in the acid Breckland soils. At Oxshott this alga formed a thick green slimy covering on the surface of the heath at various points.

The only member of Euglenineae encountered, *Euglena mutabilis*, was common in both types of soils, though more abundant in some of the acid ones. *Porphyridium aerugineum* (Rhodophyceae) occurred in two alkaline and in one slightly acid soil from Breckland; it showed the best growth in the latter.

Cyanophyceae, Diatoms, Heterokontae, and Chlorophyceae thus constitute a series in which the blue-green forms show the greatest and the green algae the least preference for alkaline soils. Closely allied species may, however,



exhibit an entirely different distribution. There is evidence that other factors may be important. Thus, the Breckland soils B (alkaline) and C (acid) have a number of species in common (cf. Table III), which implies that there may be factors common to both soils which determine the occurrence of similar forms; one of these may be the  $\text{CaCO}_3$  content (see below).

The two neutral soils (16, 17) showed a poor algal flora, which on the whole had more in common with the acid than with the alkaline soils. Blue-green algae and Diatoms were completely lacking, and the bulk of the flora consisted of Chlorophyceae. *Cosmarium cucurbita*, which was frequent in both soils, is characteristic of acid soils. A number of the species present in these two soils are also found in the acid Oxshott soil (see below).

(c) *The Relation between the  $\text{CaCO}_3$  Content of the Soil and the Algal Flora.*

Only 11 out of the 24 soils contained free  $\text{CaCO}_3$  in any recognizable quantity, but in these it varied from 90.3 per cent. (3) down to 0.13 per cent. (20). Highly calcareous soils are those from Whipsnade (2), the Gog Magog Hills (3), Box Hill (7), and to a less extent Breckland A (5). The five Breckland soils, which show progressive reduction in the amount of  $\text{CaCO}_3$ , are discussed separately below.

It is a familiar fact that the distribution of certain flowering plants is determined by the presence of  $\text{CaCO}_3$  in the soil, but little is known with regard to soil-algae in this respect. Certain data as to the nature of the algal growth on calcareous rocks are, however, summarized by Fritsch (1936, p. 210). The calcareous soils examined by me contain a number of species which are not found in the other soils. These are: *Chroococcus minutus*, *Plectonema nostocorum*, *Anabaena variabilis*, *Nostoc paludosum*, *Crinalium magnum*, *Phormidium Hieronymusii*, *Microcoleus vaginatus*, *Navicula mutica* var. *nivalis*, *N. dicephala*, *Achnanthes coarctata*, *Chlorochytrium paradoxum*, *Fernandinella alpina* var. *semiglobosa*, *Vaucheria sessilis*, *V. terrestris*, *Heterothrix quadrata*, and *Porphyridium aerugineum*. Other species, found in non-calcareous soils, are lacking, viz. *Carteria acidicola*, *Borodinella polytetras*, *Trebouxia arboricola*, *Interfilum paradoxum*, *Cosmarium cucurbita*, *Zygogonium ericetorum*, and *Vischeria stellata*. This second group contains neither Cyanophyceae nor Diatoms and only one member of Heterokontae. If a larger number of soils were examined, it might be possible to compile a list of calcicoles and calcifuges.

The algae found in the highly calcareous soil from a chalk quarry in the Gog Magog Hills (3) must be mostly calcicoles. A few, like *Hormidium nitens*, *Hantzschia amphioxys*, and *Heterothrix exilis*, are widespread, and these seem to be of no great importance as indicators of the nature of the soil. Others like *Nostoc commune*, *Microcoleus vaginatus*, *Navicula mutica* var. *nivalis*, *Fernandinella alpina* var. *semiglobosa*, *Bumilleriopsis Peterseniana*, and *Heterococcus Chodati* are more significant. The failure to find certain characteristic species in the Whipsnade soil (2) is perhaps due to the fact that no moist cultures were made.



The algal flora of the calcareous soils comprises representatives of all algal classes found in the soil, although Cyanophyceae and Heterokontae are more abundant than in any others, while the non-calcareous soils usually contain scarcely any Cyanophyceae and only a few Heterokontae. Exceptions are constituted by soils 13 and 15 which possibly contain a high percentage of exchangeable Ca which might have the same effect on the vegetation.

There is every gradation between algae that live only in the highly calcareous soils and those that occur both in the calcareous and non-calcareous soils; the latter are represented by *Hormidium nitens*, *Pleurastrum terrestre*, *Muriella magna*, *Coccomyxa dispar*, *Hantzschia amphioxys*, and *Nitzschia palea*. As regards the former group, *Phormidium Hieronymusii*, *Navicula mutica* var. *nivalis*, and *N. dicephala* have only been found in soils with a  $\text{CaCO}_3$  content of 51 per cent. and over, *Microcoleus vaginatus* in soils with 17 per cent. and over, *Chlorochytrium paradoxum* in soils with a  $\text{CaCO}_3$  content varying between 14 and 51 per cent., and *Chroococcus minutus* in soils with one varying between 0.3 and 14 per cent. *Anabaena variabilis*, *Crinalium magnum*, and *Achnanthes coarctata* were confined to the two sand-dune soils (1, 9). All the other calcareous species enumerated above occurred over a rather wide range of  $\text{CaCO}_3$ .

In addition to the sixteen species there listed, there are several others which, although occasionally found in non-calcareous soils, are nevertheless very characteristic of calcareous ones. These are *Nostoc commune*, *Phormidium foveolarum*, *Chlorococcum lobatum*, *Heterococcus Chodati*, *H. caespitosus*, *Bumilleriopsis Peterseniana*, and *Navicula atomus*. Petersen regards the last-named as nitrophilous. Since the nitrogen content of most of these soils has not been estimated, it is difficult to say whether the nitrogen content is of importance, but the  $\text{CaCO}_3$  content or the pH would appear to have a stronger influence, since in the Breckland samples the  $\text{CaCO}_3$  and pH values both decrease from soils A to G, while there is a sharp rise in the nitrogen content from soils D to G. *Navicula atomus* is found in A to D, but not in G in spite of its higher nitrogen.

*Cylindrocystis Brebissonii*, *Muriella magna*, and *Euglena mutabilis*, though found in both calcareous and non-calcareous soils, attain a maximum growth only in the latter.

#### (d) The Breckland Soils.

The five samples from the Breckland grass heath are of special interest, since they afford stages in the development of a podsol, A being the youngest and G the most mature. The depth increases and the soil becomes less calcareous and more acid from A to G (Watt, 1940, p. 50). The pH values, ranging from 8.2 to 3.7, and the  $\text{CaCO}_3$  content, varying from 17 per cent. to nil, afford a good opportunity for studying the effect of these two factors on the algal flora. The vascular plants are richest in species in B, whilst in soils C-H there is a gradual elimination of the calcicoles and less tolerant



species, but very few new species appear. The greatest change is from C to D, where there is also a change in the type of humus from mull to moor.

Thirty-six species of algae were found in these samples, of which 7 were

TABLE III. *Algae Present in the Five Breckland Soil Samples arranged according to their Preference for Calcareous and Acid Soils*

	A.	B.	C.	D.	G.
pH . . . . .	8.2	7.8	6.2	4.4	3.7
CaCO <sub>3</sub> , per cent. . . . .	17.04	1.6	0.13	nil	nil
<i>Chroococcus minutus</i> . . . . .	×	—	—	—	—
<i>Nostoc commune</i> . . . . .	×	—	—	—	—
<i>Tolypothrix tenuis</i> f. <i>terrestris</i> . . . . .	×	—	—	—	—
<i>Microcoleus vaginatus</i> . . . . .	×	—	—	—	—
<i>Pinnularia parva</i> var. <i>Lagerstedti</i> forma <i>interrupta</i> . . . . .	×	—	—	—	—
<i>Hantzschia amphioxys</i> . . . . .	×	—	—	—	—
<i>Chlorochytrium paradoxum</i> . . . . .	×	—	—	—	—
<i>Nostoc paludosum</i> . . . . .	×	×	—	—	—
<i>Heterococcus Chodati</i> . . . . .	×	×	—	—	—
<i>Plectonema nostocorum</i> . . . . .	×	×	×	—	—
<i>Phormidium autumnale</i> . . . . .	×	×	×	—	—
<i>Gloeotila protogenita</i> . . . . .	×	×	×	—	—
<i>Fernandinella alpina</i> var. <i>semiglobosa</i> . . . . .	×	×	×	—	—
<i>Bumilleriopsis Peterseniana</i> . . . . .	×	×	×	—	—
<i>Heterothrix exilis</i> . . . . .	×	×	×	—	—
<i>Porphyridium aerugineum</i> . . . . .	×	×	×	—	—
<i>Heterococcus caespitosus</i> . . . . .	×	×	×	×	—
<i>Navicula atomus</i> . . . . .	×	×	×	×	—
<i>Chlamydomonas calcicola</i> . . . . .	×	×	×	×	×
<i>Chlorococcum</i> sp. . . . .	×	×	×	×	×
<i>Interfilum paradoxum</i> var. <i>reticulatum</i> . . . . .	×	×	×	×	×
<i>Hormidium nitens</i> . . . . .	×	×	×	×	×
<i>Cylindrocystis Brebissonii</i> . . . . .	×	×	×	×	×
<i>Botrydiopsis anglica</i> . . . . .	×	×	×	×	×
<i>Chrysophyceae</i> . . . . .	—	×	×	—	—
<i>Heterothrix quadrata</i> . . . . .	—	×	×	—	—
<i>Pleurochloris</i> sp. . . . .	—	×	×	×	×
<i>Chlamydomonas</i> sp. . . . .	—	×	×	×	×
<i>Dictyosphaerium terrestre</i> . . . . .	—	×	×	×	×
<i>Chlamydomonas</i> sp. . . . .	—	—	×	×	×
<i>Macrochloris dissecta</i> . . . . .	—	—	×	×	×
<i>Muriella magna</i> . . . . .	—	—	×	—	×
<i>Hormidium crenulatum</i> var. <i>b</i> . . . . .	—	—	×	×	×
<i>Chlamydomonas subangulosa</i> . . . . .	—	—	—	×	×
<i>Euglena mutabilis</i> . . . . .	—	—	—	×	×
<i>Coccomyxa dispar</i> . . . . .	—	—	—	×	—
<i>Cyanophyceae</i> . . . . .	7	3	2	0	0
<i>Diatoms</i> . . . . .	3	1	1	1	0
<i>Heterokontae</i> . . . . .	6	7	6	2	2
<i>Chlorophyceae</i> . . . . .	7	9	13	13	12
Totals . . . . .	24	22	24	17	15

Cyanophyceae, 7 Heterokontae, 3 Diatoms, 17 Chlorophyceae, 1 Chrysophyceae, and 1 a member of the Bangiales. The distribution of the different classes in the samples is shown in Table III. The three calcareous soils are richest in algae, and there is no difference in this respect between A, which

is poor in vascular plants and bryophytes but rich in lichens, and B, which is rich in vascular plants. The decrease in number of algae in D and G runs parallel with a decrease in the number of higher plants.

The maximum number of blue-green algae occurs in A, and there is a progressive elimination of species until in D and G this class is completely absent. The Diatoms show a comparable change. The number of Heterokontae does not exhibit any appreciable differences in the first three soils, but in D and G they are strongly reduced. In all three classes no other species replace those which disappear. The Chlorophyceae, on the other hand, increase in C–G and also exhibit a change of species. Thus *Chlorochytrium paradoxum* is found only in A, while C has three species (*Gloeotila protogenita*, *Fernandinella*, and *Muriella magna*) which are not found in D, and D has three (*Chlamydomonas subangulosa*, *Coccomyxa dispar*, and *Euglena mutabilis*) not found in C, although the number of species in both samples is the same.

As Table III shows, the soil A contains a considerable number of calcicole species, as well as some that are tolerant also of non-calcareous soils. The species found exclusively in A include the two extreme calcicoles *Microcoleus vaginatus* and *Chlorochytrium paradoxum* (see p. 334) and five other calcicole species. *Hantzschia amphioxys*, which is present in most soils, was confined to A in the Breckland series.

In B the more extreme calcicoles are absent, although there are still a large number of lime-loving species; several of these are also present in C. B has no species exclusive to it, but *Nostoc paludosum* and *Heterococcus Chodati* are found in A and B only. Four species, not found in A, are common to B and C. In other words B is transitional between A and C. The algal flora of C is composed largely of the tolerant algae and is on the whole very similar to B. A few of the less particular calcicoles, like *Bumilleriopsis* and *Heterothrix exilis*, persist, while 4 species which also occur in D and G make their appearance. C has 20 species in common with B and 2 of these, viz. a member of Chrysophyceae and *Heterothrix quadrata*, are found in C and B alone.

There is a marked change in the flora of D due to the presence of forms which are typical of acid heaths, viz. *Chlamydomonas subangulosa*, *Hormidium crenulatum* var. *b*, which is dominant, and *Euglena mutabilis*. *Coccomyxa dispar* was found in this sample only. The presence of the calcicoles *Navicula atomus* and *Heterococcus caespitosus* is probably to be explained by the occurrence in D of small patches of chalky soil where these species might persist. G is similar to D, except that these two species are lacking. *Cylindrocystis Brebissonii* is the only abundant species. Except for one member of Heterokontae and *Euglena mutabilis*, all the species present belong to Chlorophyceae, a feature that is characteristic of heath-soils. It is noticeable that D and G have 6 species in common with Oxshott Heath (cf. Table II, soil 22), while A has only 2, the ubiquitous *Hormidium nitens* and *Chlorococcum* sp.

This interesting series therefore exhibits a gradual change from an algal flora judged to be characteristic of calcareous and highly alkaline soils to

that typical of the soil of acid heaths. The effect of the presence of  $\text{CaCO}_3$  in the soil in part explains the contradictory results obtained in comparing alkaline and acid soils. Since all the calcareous soils are alkaline, the calcicoles are also characteristic of alkaline soils, but some of the slightly acid soils may contain a small amount of  $\text{CaCO}_3$  (cf. Breckland C) which might determine the presence of seemingly alkaline soil-forms in acid soils.

(e) *Other Calcareous Soils.*

The Box Hill (7) soil was collected towards the top of the slope, where the low turf formed a continuous mat and probably prevented too much leaching. The surface soil was black and compact with numerous flints, and contained a high percentage of fine sand, clay, and silt (see Table IV), so that a certain amount of moisture was retained even in the hottest parts of the year. These features, together with the high percentage of organic matter and the lack of shading, evidently provide a soil very suitable for algal growth, since it has afforded the largest number of species among all the soils examined, although in nature the only prominent growth is *Nostoc commune*.

A large number of the species present are calcicoles. *Chlorochytrium paradoxum*, a marked calcicole, occurred in large numbers in the moist cultures. *Vaucheria sessilis*, *V. terrestris*, and *Pseudendoclonium basiliense* var. *Brandii* were found in this soil only. Of the 40 species present there are 20 Chlorophyceae, 8 Diatoms, 5 Heterokontae, and 1 Chrysophyceae. Though few in species, the blue-green algae are often the dominant forms, *Phormidium autumnale* and *P. foveolarum* in particular forming extensive growths on the moist cultures.

The Whipsnade (2) soil is similar to that of Box Hill in every respect, although containing a higher percentage of  $\text{CaCO}_3$ . The flora was incompletely studied, but of the 19 species found 14 are common to Box Hill, while the proportions of species belonging to the different algal classes is roughly the same. The only striking difference is the presence of *Tolypothrix tenuis* forma *terrestris*. Soil (3), from the Gog Magog Hills, is again remarkably similar to that of Box Hill, sharing 18 of the 21 species with the latter. The chief contrast lies in the presence of a different member of Chrysophyceae and of *Horridium crenulatum* var. *a*.

(f) *Soils from Sand-dunes.*

The algal flora of the two soils from sand-dunes near Llandudno, and especially that on the loose sand (9), is similar to that of the chalk soils, as shown by the presence of *Chroococcus minutus*, *Nostoc commune*, *Heterothrix exilis*, *Bumilleriopsis Peterseniana*, and *Fernandinella alpina* var. *semiglobosa*; the last two, as well as the species of *Phormidium* and the *Nostoc*, are absent from the firmer sand (soil 1), which may in part be due to competition with higher plants (Piercy, 1917, p. 515). *Crinalium magnum* and *Achnanthes coarctata* were found in these soils only, though the latter is known to be a frequent soil form.



(g) *Non-calcareous Alkaline Soils.*

The soil collected from sloping grassland near Loch Doon (6), though containing small traces of calcium carbonate, has certain features in common with the non-calcareous soils. Such are the large number of palmellaceous forms and the presence of *Eunotia tenella* which is characteristic of acid soils. A few calcicoles are present. *Cylindrospermum alatosporum*, *Anabaena torulosa*, *Euastrum sublobatum* var. *subdissimile*, and several species of *Cosmarium* were found only in this soil. The presence of nine species of Desmids is an outstanding characteristic.

Among the 34 species identified, there are 19 Chlorophyceae, 6 Cyanophyceae, 8 Diatoms, and 1 Heterokontae. The large number of green algae, and the presence of only one member of Heterokontae and that a Pleurochloris, indicates the similarity with other non-calcareous soils, while the presence of a number of blue-green algae, some of which are calcicoles, is a feature of calcareous soils. The Loch Doon soil therefore shows features common both to the calcareous and the non-calcareous soils.

Two samples of soil taken from the sedge-area at Wicken Fen (12) in September and November respectively both contained the same species. Some of the algae present, like *Oedogonium* sp., are aquatic forms. This soil differs from all the others, except that from Skegness (15), in the predominance of Diatoms. There are 7 Chlorophyceae, 2 Cyanophyceae, 3 Heterokontae, and a member of Chrysophyceae which also occurred in soil (19). *Amphora Normani*, *Diploneis elliptica*, and *Cosmarium etchachanense* were confined to this soil.

(h) *Acid and neutral soils.*

(1) *Heath soils.* The soil of the south ridge (Summerhayes, Cole, and William, 1924) of the *Calluna*-heath at Oxshott (22) is very sandy and acid (Table IV). Similar highly acid grass heath soils are samples D (23) and G (24) from Breckland. On the other hand, those from a heath near Bournemouth (17) and from a pine-wood near Loch Lomond (16) are neutral.

The heathland soils are for the most part characterized by the comparatively few species present, although this is not quite true of the Oxshott soil with twenty species and in the case of the two neutral soils the paucity of species may be due to shading by trees. Blue-green algae are absent from all the heathland samples, while Diatoms are very rare and the Heterokontae represented only by a single species in each soil, never common except *Vischeria stellata* in the older cultures of soil (16). The bulk of the algal flora is made up of unicellular Chlorophyceae, with *Hormidium nitens*, *H. crenulatum* var. *b*, and occasionally *Interfilum paradoxum* and *Stichococcus bacillaris*.

While the Heterokontae present in the five samples vary, the Chlorophyceae show a considerable degree of uniformity. The two neutral soils (16 and 17) have five species of green algae in common and all of them, except for



the species of *Chlamydomonas*, are also present in the Oxshott soil. The neutral heaths differ from the latter in the absence of certain species (including the acid-loving ones). The Breckland soils, on the other hand, present certain differences; *Cylindrocystis Brebissonii* takes the place of *Cosmarium cucurbita*, while *Macrochloris dissecta* and *Dictyosphaerium terrestre* were found only in them.

TABLE IV. *Soil Analyses*

	Epping.	Oxshott.	Box Hill.	Widcombe.
pH . . . . .	5.2	4.0	8.0	6.6
Hygroscopic moisture, per cent.	6.0	1.7	4.04	2.0
Organic matter, per cent. . .	10.17	5.72	14.0	11.5
Salts, per cent. . . . .	4.5	2.96	4.26	6.7
Clay, per cent. . . . .	5.05	2.63	3.5	4.75
Silt, per cent. . . . .	7.45	0.38	0.75	4.0
Fine sand, per cent. . . . .	55.63	85.46	58.01	45.46
Coarse sand, per cent. . . .	11.2	1.16	6.59	25.6
CaCO <sub>3</sub> , per cent. . . . .	nil	nil	51.26	nil

Some of the characteristic algae of heath soils are: *Chlamydomonas subangulosa*\*, *Carteria acidicola*\*, *Borodinella polytetras*\*, these two confined to Oxshott, *Chlorococcum lobatum* var. *tenue*, *Trebouxia arboricola*\*, *Dactylococcus bicaudatus*, *Chlorella vulgaris*, *Muriella magna*, *Coccomyxa dispar*, *Interfilum paradoxum*, *Hormidium crenulatum* var. *b*, *Pleurastrum terrestre*, *Cosmarium cucurbita*\*, and *Cylindrocystis Brebissonii*. Those marked with a \* are confined to heath soils and may probably serve as indicator species, although the general composition of the algal flora is a surer indication of the nature of the soil.

(2) The acid soil (21) from the mixed wood in Epping Forest resembles the Oxshott soil (see Table IV) in the low pH, the absence of lime, and the high proportion of fine sand, but the percentage of salts is higher, there is more silt and clay, and there is deep shade. The vegetation is classed as a heathy beech wood formation by Tansley (1939, p. 408). The algal flora is very similar to that of the Oxshott soil.

Cyanophyceae are again absent, the rare Diatoms are represented by *Hantzschia amphioxys* and *Pinnularia borealis*, and the Heterokontae by *Pleurochloris acidophila*. Thirteen species of Chlorophyceae are common to both soils, but that from Epping lacks *Carteria acidicola*, *Chlamydomonas subangulosa*, and *Cosmarium cucurbita*. *Zygogonium ericetorum* was present in both, although not necessarily at the place of collection.

(3) The sandy soil (11) bearing a growth of bracken and covered with dead organic matter, though faintly alkaline, shows resemblances to the heath soils and like (17) afforded only a few species. It differs from the heath soils in containing *Nostoc humifusum*, which appeared in the moist cultures after about a year. The only Diatom was *Pinnularia borealis*, while the green algae were represented by *Hormidium nitens*, *Chlorococcum* sp., *Chlamydomonas*

*pseudoelegans*, and *C. minutissima*. The last two were later replaced by *Polyedriella helvetica*, an alga also found in the alkaline soil from Gaydon (4). The only other Heterokontan present was *Heterothrix exilis*.

(4) The only moorland soil examined was one from Dartmoor (19), rich in peat. By contrast to the heath soils this contains *Phormidium autumnale*, while four species of Diatoms are present. The Heterokontae comprise *Botrydiopsis anglica* and *Heterothrix exilis*, both common to a variety of soils. Characteristic of this soil is *Chlamydomonas pseudogloeogama* which formed small macroscopic colonies in the moist cultures. Three Desmids are present, and it is noteworthy that this is the only soil in which *Cylindrocystis Brebissonii* and *Cosmarium cucurbita* were found side by side. A member of Chrysophyceae, found also in Wicken Fen (12), was common in the moist cultures. The presence of a large number of species with considerable mucilage-production was a striking feature of the liquid cultures from this soil.

#### IV. DIFFERENCES BETWEEN THE LIQUID AND MOIST CULTURES

The conditions in the liquid cultures are different from those in nature, and certain forms, like *Zygogonium ericetorum*, do not long survive transference to such cultures. Since Benecke solution was added to the liquid cultures, the concentration of salts too was greater than that available in the natural environment. The algae that flourish in the liquid cultures may, therefore, in part be such as would play no considerable rôle in the soil. If wind-blown spores of hydrophytic species are present, these might grow well in the liquid cultures, although in nature they might remain dormant (Fritsch, 1936, p. 197). Liquid cultures do not, therefore, probably give a true picture of the algal flora, though useful as a means of finding out what algae are present.

The moist cultures (see p. 325) are a better imitation of natural conditions. They were kept in an unheated greenhouse exposed only to natural illumination. Macroscopic growths appear on slides laid on the soil, on the sides of the dish, and on the surface of the soil itself, usually after a month in summer and after two or three months in winter. Certain older cultures were allowed to dry for about two months and were then again moistened; this probably corresponds to what frequently occurs under natural conditions. Previous workers, especially Petersen, have made use of this method of culture, but the latter does not specify which algae were obtained in this way. Esmarch (1914) obtained mostly blue-green algae and no Diatoms. The drawbacks are that some of the rarer forms are probably overlooked, especially in cultures developing a considerable growth of moss protonema. Moreover, identification is often difficult, since, if the cultures are relatively dry, the cells are filled with refractive globules; this can only be satisfactorily overcome by making sub-cultures.

Good growths were obtained from all the moist cultures, except for a few from Oxshott and Epping which became covered with fungi and bacteria. Compared with conditions in nature, the moist cultures afford plenty of

moisture and absence of a cover of higher plants, and these advantages evidently have a great influence on the growth of the algae (cf. Petersen, 1935, p. 15). With few exceptions the different classes exhibit a comparable behaviour in the two sets of cultures. The subsequent matter (cf. also Table II) deals only with those instances in which an alga has been consistently more abundant in or absent from cultures of the one type.

*Cyanophyceae*. A number of these (*Nostoc commune*, *Phormidium foveolarum*, *P. autumnale*, *Microcoleus vaginatus*) formed macroscopic growths on the soil at the time of collection, which indicates their liking for terrestrial conditions. More than two-thirds of the species grew better in the moist than in the liquid cultures; two (*Phormidium tenue*, *Plectonema nostocorum*) grew equally well in both, while three (*P. foveolarum*, *P. Hieronymusii*, *P. autumnale*) were more abundant in the liquid cultures. Among the Cyanophyceae, therefore, it is essentially species of *Phormidium* that show a preference for these cultures. Species never observed in them were *Cylindrospermum* spp., *Anabaena* spp., *Nostoc humifusum*, *Phormidium uncinatum*, *Oscillatoria formosa*, and *Schizothrix arenaria*.

*Heterokontae*. All were found in both cultures, but most were commoner in the liquid cultures. *Vischeria stellata* and *Polyedriella helvetica*, however, were more prominent in the moist cultures, while *Pleurochloris terrestris* was equally represented in both.

*Diatoms*. These grew better in the moist than in the liquid cultures. Of the 23 species and varieties observed, only *Navicula mutica* var. *nivalis* exhibited better growth in the liquid cultures, while 17 developed better in the moist ones. No species was absent from the moist cultures, although a considerable number did not appear in the liquid ones. Most of these are, however, known to occur in aquatic habitats, which suggests that special terrestrial forms or races may be involved. It will be evident that no list of soil Diatoms compiled from liquid cultures alone can be regarded as complete.

*Chlorophyceae*. The two *Carterias* grew well in both types of cultures, while the species of *Chlamydomonas* were much commoner in the liquid than in the moist cultures, except for *C. minutissima* and *C. pseudogloeogama* which developed better in the latter. All the Chlorococcales were found in both cultures and most grew better in the liquid ones; only *Coccomyxa dispar* developed equally well in both. Except for *Hormidium crenulatum* var. *a*, which was found only in the moist cultures, all the Ulotrichales developed better in the liquid ones, and this was also true of *Pleurastrum terrestre* and *Pseudendoclonium basiliense* var. *Brandii* (Chaetophorales). The *Pleurastrum* was sometimes, however, observed in small amount on the freshly collected soil. The two *Vaucherias* never appeared in the liquid cultures.

The Desmids were more abundant in the moist cultures and only *Closterium pusillum* var. *monolithum*, *Cylindrocystis Brebissonii*, and *Cosmarium cucurbita* appeared in the liquid ones. The two species last named formed extensive



growths on the surface of the moist cultures and exhibited conjugation when they began to dry up. *Cosmarium cucurbita* can withstand intense desiccation. After three months' drying the individuals showed shrunken protoplasts, but three days after addition of water normal individuals were again present. *Zygonium ericetorum*, which is common in the moist cultures, is represented only by stray filaments in the liquid ones.

The green algae thus fall into two groups: (1) those which grow better in the liquid cultures, comprising Chlamydomonas, the Chlorococcales, Ulotrichales, and Chaetophorales; (2) those which grow better in the moist cultures, comprising the Vaucheriaceae, Desmids, and *Zygonium ericetorum*.

*Euglena mutabilis* grew well in both cultures, but was usually more abundant in the moist ones. The few colourless Euglenineae observed were found only in the liquid cultures. *Porphyridium aerugineum* was commoner in the liquid than in the moist cultures.

#### V. DIFFERENCES BETWEEN THE ALGAL GROWTH AT SUCCESSIVE DEPTHS IN THE SURFACE SOIL

As already mentioned (p. 324) samples were taken at successive centimetre depths from the soils at Epping, Oxshott, and Box Hill. Moist cultures were made only of the two uppermost centimetre samples and only occasional reference is made to them. The liquid cultures were examined when the first signs of growth became apparent, again after a month, and sometimes also six months or a year later. Since there is little difference between the two upper and the two lower layers respectively, they are mostly considered together, i.e. distinction is made between an upper layer down to 2 cm. and a lower one between 2 and 4 cm.

The more constant species found fall into three groups, viz. (1) those that are commoner in the upper than in the lower layer, (2) those that are commoner in the lower layer, and (3) those that are equally common in both.

(1) *Epping*. The green algae, which are in the majority, for the most part belong to group 3. The common forms are *Chlorococcum humicolum*, *Muriella magna*, and *Coccomyxa dispar*. The Trochiscia-stage of *Chlorococcum* (see Part II) was more often found in the lower layers. The only algae in group 1 are *Euglena mutabilis* and *Hormidium nitens*, of which the former shows a distinct preference for the upper and was found only once in the lower layer; in the moist cultures it was always more frequent in the top than in the second centimetre, and this marks it out as a distinct surface-form. The *Hormidium* was often abundant in the actual surface layer, less frequent in the second centimetre, while in the two lower layers it was rare or absent. There are no algae in this soil belonging to group 2, although *Pleurochloris acidophila* was more often found in the lower than in the upper layers.

(2) *Oxshott*. The dominant green algae again belong mostly to group 3, although *Muriella magna* and *Pleurastrum terrestre* were occasionally commoner in the upper layer. Both produce an orange-coloured oil and can



withstand a good deal of desiccation. Since the soil is sandy and exposed to winds it becomes very dry at times, so that only those algae which are adapted to withstand drought can live at the surface. These factors may in part account for the paucity of species in group 1. *Euglena mutabilis*, which in the moister, shaded soil of Epping belongs to group 1, here showed no preference for the surface, occurring almost equally in both layers and occasionally even being commoner in the lower layers. This difference in the distribution of the same alga in the two soils is of interest and implies that *E. mutabilis* is a shade-loving form, unable to withstand much desiccation.

The only alga showing any marked preference for the lower layer was *Pleurochloris acidophila*, the only member of Heterokontae present; in 4 out of the 7 collections it was commoner in the lower layer and the contrary was never observed. *Chlorococcum lobatum* var. *tenue* showed a slight preference for the lower layer and again the Trochiscia-stage was more frequent there, but the difference is not so marked as in the Epping soil.

(3) *Box Hill*. This soil proved to be very suitable for a study of microstratification, as it contains a wide range of species. Like that at Oxshott it is exposed to insolation and desiccation, but retains more moisture because of its clayey texture and the presence of a covering of grass. The second centimetre layer here seems to be intermediate in character between the surface and the two lower layers.

Group 1 is well represented, but consists only of blue-green algae. *Phormidium foveolarum* is common in the surface layer, but either rare or absent in the lower layers. *P. autumnale* was commoner near the surface in 4 out of the 7 collections; in February it was equally common in both layers, whilst in June, when the soil was very dry, it was commoner in the lower layers. *Nostoc commune* was not often met with in the cultures, but when present was much commoner in the surface layers.

Group 2 includes several Heterokontae and Chlorophyceae, but no Cyanophyceae. *Heterococcus Chodati* was commoner in the lower layers 4 times out of 7, being otherwise equally common in both. Other Heterokontae belonging to group 2 are Bumilleriopsis, *Pleurochloris* sp., and to a less extent *Heterothrix exilis*, the last occurring commonly in all layers, though occasionally showing a preference for the lower ones. Among green algae *Chlamydomonas calcicola* and *Chlorochytrium paradoxum* belong to this group, the latter in spite of the fact that *Phormidium foveolarum*, amid the weft of which it usually occurs, belongs to group 1. Of the Diatoms, *Navicula atomus* and *Nitzschia palea*, and occasionally *Pinnularia borealis*, are commoner in the lower layers.

Group 3 consists mainly of green algae, some of the more constant species being *Hormidium nitens*, *Chlorococcum lobatum*, and *Pleurastrum terrestre*, although the second species is occasionally more frequent in the lower layers. *Hantzschia amphioxys* is the only Diatom belonging to this group.

The diverse data show that microstratification depends not only on the

kind of soil but also on the composition of the algal flora. A soil, like that of Box Hill, that contains representatives of diverse classes, shows a more marked stratification than one the flora of which is more monotonous. The blue-green algae, so far as this investigation is concerned, belong without exception to group 1. Since they are known to be able to withstand desiccation and many thrive at higher temperatures, they appear to be specially adapted to be surface-living algae. Their prevalent filamentous form probably prevents washing downwards by rain, and this may account for their lesser abundance in the lower layers. Spores were never seen in *Nostoc commune* during the two years of observation.

All the Heterokontae, except *Heterothrix exilis*, are commoner in the lower layers than at the actual surface. Fritsch (1936, p. 201), commenting on the considerable number of Heterokontae recorded in the subterranean algal flora, suggests that their non-discovery at the soil-surface may be due to their normal habitat being just below the surface, since members of this class are known to favour weaker illumination. This suggestion has been confirmed in the three soils above considered. In this connexion it is of interest to note that at Epping, where there is a considerable amount of shade, *Pleurochloris acidophila* is more evenly distributed in the different layers than at Oxshott. *Heterococcus Chodati* (*H. viridis*), which has so far been regarded as a typical subterranean form, was one of the algae found in the lower layers of the surface-soil. This tends to support the view that the subterranean algal flora is derived from the surface-community.

Fritsch (1936, p. 204) likewise suggests that common surface-forms, like *Hormidium*, *Chlorococcum*, *Stichococcus*, &c., may tide over periods unfavourable for growth at the actual surface by persistence in the layers just below the surface. On the return of favourable conditions (wet weather), such persisting individuals would help to form a macroscopically visible growth at the surface. Some support is given to this hypothesis by the fact that *Hormidium nitens*, which at Epping is usually commonest in the actual surface-layer, in the hot dry month of June was found to be more common in the second centimetre layer. Moreover, this species shows no preference for the surface in the more exposed soils of Oxshott and Box Hill. *Phormidium autumnale*, which is a marked surface-form at Box Hill, was also more common in the second and third centimetre layers in June.

It is noticeable that the Diatoms, with the exception of *Hantzschia amphioxys*, are commoner below than at the actual surface, although their number is so small that no general conclusions are possible. Attention may be drawn to the fact that most of the green algae belong to the third group.

## VI. SEASONAL VARIATION

Samples were collected once a month over a period of nearly two years from the three soils discussed in the previous section. The freshly collected samples were examined immediately after collection and disclosed a limited

number of algae, but since it is very difficult to recognize forms present in small quantity by mere inspection of the surface, many will have been overlooked. The growth, appearing in the moist cultures during the first month, gives a better indication of the algal flora present, although there are evident sources of error. Thus, *Nostoc commune*, though often quite common at the time of collection, seldom appeared in the fresh cultures. The change from nature to laboratory conditions no doubt affects certain forms by killing the vegetative cells, although resting stages may survive and lead to the reappearance of the alga in the culture at a later stage.

This is specially true of liquid cultures in which most of the species, found in the soil at the time of collection, were absent or rare during the first month after growth started, although most of them occurred commonly at some later stage. The growth in these cultures does not, therefore, give as good an indication of that in the soil at the time of collection as that in moist cultures. It must be realized, however, that such cultures, undertaken under laboratory conditions at different times of the year, are not altogether comparable, since factors like temperature and illumination will have varied to a marked degree.

(1) *Epping*. *Zygonium ericetorum* was in general present all the year round and becomes common in spring; in 1939 it was abundant from February to April, although in 1938 when it was drier it flourished only for a short time in March. It was common in the moist cultures from December to March. Other species are rare in the freshly collected soils and only occur occasionally. *Chlorococcum humicolum*, *Hormidium nitens*, and *Hantzschia amphioxys* are met with in wet weather, while *Pleurastrum terrestre* was found very occasionally in spring, but from July to December only *Zygonium* was present.

In the moist cultures the best growth during the first month after algae began to appear was obtained in samples collected between January and March, and the poorest from those gathered between July and October. *Euglena mutabilis*, *Zygonium ericetorum*, *Chlorococcum humicolum*, and occasionally *Coccomyxa dispar* were common. The first was absent from April to December 1938, and it would seem that a moist soil and possibly the absence of shade from the trees favour its growth.

(2) *Oxshott*. The only visible growth on this very dry and sandy soil consisted of small and inconstant numbers of *Trebouxia arboricola*, *Chlamydomonas* sp., and *Euglena mutabilis* found in wet weather. In the first month after growth started in the moist cultures *Carteria acidicola*, *Chlamydomonas* sp., and *Stichococcus bacillaris* were sometimes common. There was usually an increase in the number of species from October to February or April and then a decrease, coinciding with the hot season.

(3) *Box Hill*. The freshly collected soil contained the most abundant growth from November to February or April. This consisted of *Nostoc commune*, *Phormidium foveolarum*, *P. autumnale*, *Microcoleus vaginatus*, *Chlorococcum* sp.,



*Navicula atomus*, *Pinnularia borealis*, *P. parva* var. *Lagerstedtii* forma *interrupta*, and *Hantzschia amphioxys*. Judging from the moist cultures, species of *Nostoc* are rare or absent in autumn, while *Phormidium foveolarum* and *P. autumnale* are practically always present. The *Vaucherias* seem to be winter- or early spring-forms, which are absent in the hotter and drier parts of the year. *Euglena mutabilis* was found only in April of both years. Heterokontae were seldom found in the freshly collected soil, but in the moist cultures *Botrydiopsis anglica*, *Heterothrix exilis*, and *Heterococcus Chodati* appeared as rare forms. All were absent from June to December. The best growth of *Navicula atomus* and *Pinnularia parva* var. was observed in spring and early summer months.

In these soils, therefore, growth is evident at the time of collection only during the wetter months and in general during those of winter when the light is not intense. That persisting individuals or dormant stages that readily germinate are nevertheless present at other times of the year is shown by the data obtained from the moist cultures. Special investigation by other methods would, however, be necessary to determine whether the few indications of a periodical larger development of certain forms in the soil at these times of the year really obtains or whether such apparent periodicity is not a result of the varying conditions affecting the moist cultures. There are indications (see previous section) that certain soil-forms are at times less common at the actual surface than at a few centimetres beneath it.

#### VII. THE SUCCESSION OF ALGAL GROWTH IN CULTURES MAINTAINED FOR A NUMBER OF MONTHS

In those liquid and moist cultures, which were examined at intervals for about a year, there is a definite succession, which is the same whether the cultures are started in winter or summer. In general, blue-green algae are the last to appear; *Phormidium foveolarum* and *P. autumnale*, present in small quantities in fresh cultures, became common only three or four months after growth had started. Most Cyanophyceae are found within six months, but some (*Nostoc commune*, *Chroococcus minutus*) only appear at a much later date, sometimes not until two years have elapsed. In old cultures blue-green algae are dominant, the other forms having disappeared or being greatly reduced in number.

On the other hand, most of the green algae grow better in the fresh cultures and species of *Chlamydomonas* and *Chlorococcum* are among the first to appear. Exceptions are constituted by *Chlorochytrium paradoxum* and *Coccomyxa dispar*, found in most of the old cultures, and *Cosmarium cucurbita*, which sometimes formed a dense covering on the older moist cultures. Most of the Heterokontae occur only in the fresh cultures. *Heterococcus* was never found in cultures more than three months old, while *Botrydiopsis anglica*, *Bumilleriopsis Peterseniana*, and *Heterothrix exilis* sometimes persist for as long



as six months. It is impossible to induce a fresh growth of these forms in old cultures by the addition of further nutritive solution. Species of *Pleurochloris*, however, usually appear in old liquid cultures, while *Vischeria stellata* and *Polyedriella helvetica* are characteristic of old moist cultures. Diatoms attain their maximum development about four months after the cultures are started and have usually all disappeared after a year. Chrysophyceae occur only for a very short time and are seldom present until three or four months have elapsed.

TABLE V. *Number of Species belonging to Different Classes in the Breckland Soil-cultures at Successive Stages*

No. of months cultured.	Heterokontae.					Cyanophyceae.					Chlorophyceae.				
	A.	B.	C.	D.	G.	A.	B.	C.	D.	G.	A.	B.	C.	D.	G.
1	2	4	3	1	0	0	0	0	0	0	0	3	5	8	0
2	5	6	4	1	1	2	0	1	0	0	3	7	11	10	7
4	2	3	3	0	1	4	1	2	0	0	5	7	9	11	10
6	0	1	0	0	0	7	3	2	0	0	3	6	4	9	4

A well marked succession is seen especially in cultures of chalk soils. The first to develop are the Heterokontae, accompanied by *Chlamydomonas* and a few green cells. These increase in number, whilst other green algae (*Hormidium*, *Chlorococcum*), as well as Diatoms and threads of *Phormidium*, begin to appear. Meanwhile the Heterokontae decrease in number and disappear, except for *Pleurochloris* and occasionally *Heterothrix exilis*. The Chlorophyceae persist, while Diatoms and Cyanophyceae increase. In the next stage the latter become dominant and most of the green algae disappear, although many can be induced to grow again in the liquid cultures by the addition of fresh nutritive solution. In cultures of acid soils, on the other hand, there is no marked sequence. The first algae to appear are usually species of *Chlamydomonas* and these are followed by other green forms, among which *Coccomyxa* becomes abundant in the older cultures.

Table V shows the number of species of the different classes found in the Breckland cultures at successive stages. This illustrates clearly the change in the composition of the flora of the calcareous soil A with age, while in the acid soils D and G there is no such clear sequence. Since all these cultures were started in December, the change in the algal flora in this instance also coincides with an increase in illumination and temperature.

### VIII. SUMMARY

The composition of the algal flora inhabiting the surface-layers of a number of uncultivated soils is investigated. The pH of the soil is found to be an important factor in determining the type of algal flora present. Cyanophyceae, Diatoms, and Heterokontae grow better in alkaline soils, while Chlorophyceae are better represented in the acid ones. The effect of the amount of  $\text{CaCO}_3$  in the soil is considered and a tentative list of calcicole and calcifuge species

drawn up. The calcareous soils possess a characteristic algal flora. Five soil-samples from Breckland grass heath representing stages in the formation of a podsol show very clearly the influence of decreasing  $\text{CaCO}_3$  and of the change from an alkaline to an acid reaction on the composition of the algal flora. Acid heaths have an algal flora composed almost wholly of green algae.

The algae found can be grouped into (1) those which grow well only in moist cultures and are specially adapted to terrestrial life, and (2) those which grow better in liquid cultures. Almost all the Cyanophyceae, most of the Diatoms, and some of the Chlorophyceae, develop better in moist cultures, while almost all the Heterokontae and the remainder of the Chlorophyceae are more abundant in liquid cultures.

The occurrence of a microstratification of the algal flora in the surface layers, first suggested by Fritsch, is confirmed. Certain algae are characteristic of the upper, while others (specially Heterokontae) are commoner in the lower layers. Under unfavourable conditions some of the usual surface-living forms become more frequent in the lower layers. In nature the best growth is observed in the cooler, wetter months, while in cultures it is usually found in early spring.

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# Statistical and Ecological Studies in the Distribution of Species in Plant Communities

## I. Dispersion as a Factor in the Study of Changes in Plant Populations

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With ten Figures in the Text

### INTRODUCTION

IN an earlier paper (Blackman, 1935) a statistical examination was made of some of the methods commonly employed for the estimation of changes in the botanical composition of grassland communities. It was shown in the case of tiller-frequency counts or of estimates of the area covered that the data obtained suffered from the disadvantage that the frequency distribution curves were markedly skew and therefore the statistical errors could not readily be calculated. It was concluded that the estimation of percentage absence, i.e. the percentage number of sample quadrats which do *not* contain individuals of the species under investigation, has much to recommend it. Firstly, such an estimate can be readily and quickly obtained. Secondly, there is little or no personal bias attached to the method as is the case with others which call for judgement on the part of the observer, e.g. percentage area covered (West, 1938), subjective frequency estimates (Hope Simpson, 1940).

Besides, however, the advantages of quickness and lack of bias, changes in percentage absence can be related to changes in density. If individuals of a species are distributed at random in the community under examination, then the number of sample quadrats which contain nought, one, two, three, &c., plants can be evaluated from the discontinuous Poisson distribution, namely

$$e^{-m}, me^{-m}, \frac{m^2e^{-m}}{2!}, \frac{m^3e^{-m}}{3!}, \dots, \frac{m^ne^{-m}}{n!},$$

where  $m$  is the mean density per quadrat. It follows therefore that if the species is distributed at random, the mean density can be calculated from the equation

$$\text{percentage absence} = 100e^{-m}.$$

Where it is possible to identify individual plants of a species the randomness

of the distribution can be tested by fitting from the mean density estimate a Poisson distribution and applying the  $\chi^2$  test. Further information can be obtained by calculating the variance of the density  $m$  and determining the ratio this variance bears to the mean density, i.e.  $\sum \frac{(m-\bar{m})^2}{(n-1)\bar{m}}$ . In the case of

the Poisson distribution this ratio is unity and departure from unity is a measure of dispersion. Where the value exceeds unity the plants are said to be over-dispersed, i.e. aggregated together. If, on the other hand, the ratio is significantly less than one, then the plants are under-dispersed, e.g. assume a pattern as if the individual plants were mutually repellent.

This estimate of dispersion, which might be termed a 'reduced index of dispersion', since it is the index of dispersion divided by the degrees of freedom, has been called by Clapham (1936) 'relative variance'. Relative variance has, however, been used by others in a different sense, namely, to denote the variance divided by the *square* of the mean.<sup>1</sup> In order therefore to avoid confusion it is proposed to substitute in the present paper 'coefficient of dispersion' for 'relative variance' as defined by Clapham.

The estimation of the dispersion of plants was first carried out in the field by Svedberg (1922), but his estimates were for a number of species based on counts of inflorescences rather than the more satisfactory data of plant numbers. Ashby (1935) applied a test devised by Stevens to determine significant departures from a random distribution to counts of *Salicornia*. Clapham (1936) examined statistically data obtained by Steiger (1930), who in two prairie communities determined the number of individuals of each species in forty quadrats. Clapham found that in these two communities the majority of species were over-dispersed (aggregated), since in forty out of forty-four species the coefficient of dispersion (relative variance) was greater than unity and reached in the case of *Poa pratensis* a value of 37.54. Clapham also showed that, because of this aggregation, the mean densities calculated from the percentage absence data did not for most species agree with the actual densities observed in the field by Steiger. On the other hand, it had been shown in a previous paper (Blackman, 1935) that a number of species in different grassland communities were distributed at random, and, moreover, that, in the case of *Plantago media*, in spite of the distribution not being at random but over-dispersed, the relationship between the logarithm of percentage absence and mean density was approximately linear over a wide range of densities. In consequence it was one of the objects of the present series of investigations to determine whether in spite of over-dispersion other species showed the same linear relationship, for, if this were so, then changes in density could still be measured relatively, although not absolutely, from changes in the percentage absence estimate. The second and more fundamentally important object was to discover to what extent the coefficient of dispersion was dependent upon the species, and to what extent it was

<sup>1</sup> Information supplied in correspondence by Professor R. A. Fisher.

influenced by environmental conditions. The present paper is mainly concerned with the first object.

### EXPERIMENTAL RESULTS

#### *Communities examined.*

The data of this investigation fall naturally into two classes : those obtained for alpine meadows in the Tyrol and those obtained for chalk downland in England. In the case of the alpine meadows a comparison was made of the distribution of a few species in communities widely distant from each other but having in common the same dominant plant, *Nardus stricta* (*Calluna vulgaris* sometimes sub-dominant). In the downland communities stress was laid rather on differences in distribution between adjacent plots within the same community; in all such communities the dominant grasses were *Festuca ovina* and *F. rubra*.

#### *Statistical Methods.*

In the statistical treatment of the present data both the coefficient of dispersion and  $\chi^2$  tests have been applied. Although these tests both detect significant discrepancies from a random Poisson distribution, they are to a certain extent complementary. In the case of the coefficient of dispersion test, if the value is greater or less than unity by  $2\sqrt{\left(\frac{2n}{(n-1)^2}\right)}$  ( $n$  = number of sample quadrats), then it can be considered that the distribution is not a random one. When the coefficient of dispersion value is not significantly different from unity the  $\chi^2$  test should still be applied, since the coefficient of dispersion test, although sensitive as regards aggregation, will not detect certain types of skew distribution. For example, in the present investigation it has been found that if the sum of the positive departures of the observed frequencies from the calculated is approximately equal to the sum of the negative departures, then this skewness from a random distribution will be detected by the  $\chi^2$  test, though the value of the coefficient of dispersion may show no significant departure from unity, e.g.

#### *Frequency Classes (Plants of Poterium Sanguisorba per Quadrat)*

	0	1	2	3	4	5
Calculated . . .	44.08	36.08	14.80	4.06	0.84	0.14
Found in field . .	51	22	21	6	0	0
Difference . . .	+6.92	-14.08	+6.20		+0.96	

Sum of  $\chi^2 = 9.34$ .  $\therefore$  Value of  $P$  ( $n = 2$ ) is less than 0.01.

Coefficient of dispersion =  $\frac{\text{variance}}{\text{mean}} = \frac{92.76}{99 \times 0.82} = 1.142$ , and is therefore not significantly different from unity since it is less than 1.286;

i.e.  $1 + 2\sqrt{\left(\frac{2n}{(n-1)^2}\right)}$  for  $n = 100$  is 1.286.

*Distribution of Species in Alpine Meadows—Nardetum.*

*Distribution of Gentiana acaulis.* The distribution of *G. acaulis* was examined in fifteen different Nardetum communities. In Table I it is seen that for seven localities the coefficient of dispersion is not significantly different from unity. In five cases the  $\chi^2$  test confirms that the distribution

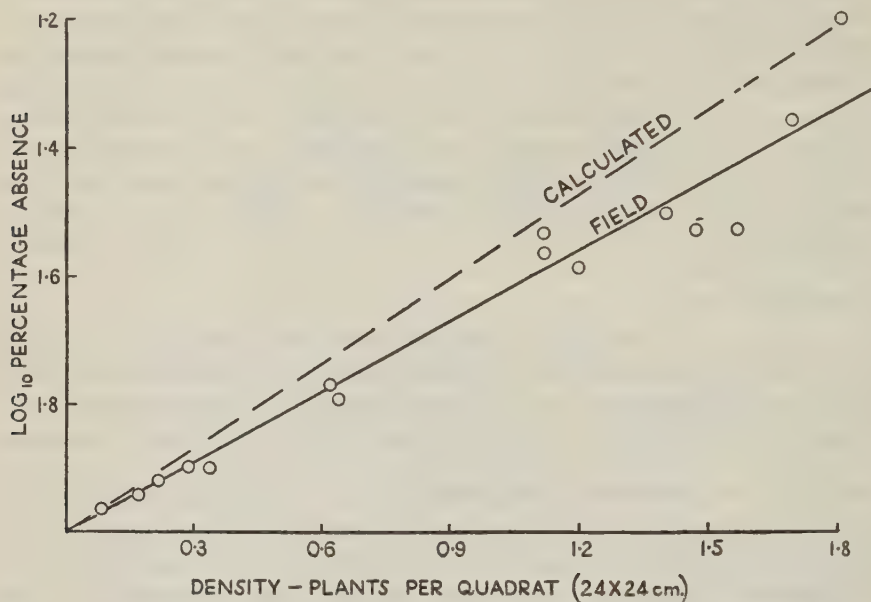


FIG. 1. *Gentiana acaulis*. The relationship between the logarithm of percentage absence and mean density. The regression calculated on the basis of a random distribution is shown as a broken line.

is at random. In the remaining two examples it is not possible to apply the  $\chi^2$  test on account of the low densities and the small number of classes available for comparison between the observed and fitted distributions. In both examples, when allowance is made for grouping in the calculated distributions the classes with values of less than five (see p. 353, Fisher, 1932, p. 86), there remain only two classes for comparison and two degrees of freedom. In consequence, since the mean of the distribution is an estimated mean, two degrees of freedom must therefore be subtracted from the total degrees of freedom, and it is not possible to apply the  $\chi^2$  test.

In the remaining eight sample areas the plants are over-dispersed, the greatest aggregation occurring in two localities at Kuhtai. In spite, however, of the variation in the coefficient of dispersion, the relationship between the logarithm of percentage absence and of mean density is approximately linear<sup>1</sup> (see Fig. 1). Since the trend in the various communities is towards over-

<sup>1</sup> The method of calculating the regression line has been given in a previous paper (Blackman, 1935).



dispersion, the linear regression, as would be expected, falls below that postulated on the basis that in all the communities the distribution is random.

TABLE I. *Distribution of Gentiana acaulis in Alpine Meadows*

Plot size: 10 × 10 m. Quadrat size: 24 × 24 cm.

Locality.	Altitude (metres).	Number of quadrats per plot.	Mean density, plants per quadrat.	Coefficient of dis- persion.*	$\chi^2$ test value of <i>P</i> .
Saint-Anton am Arlberg (i)	1600	100	0.64	1.405	—
" " (ii)	1650	100	0.29	1.344	—
" " (iii)	1700	100	1.81	1.113	0.8
" " (iv)	1750	100	0.62	1.264	0.05
" " (v)	1750	100	1.40	1.457	—
Kuhtai (i)	2000	100	0.34	1.736	—
" (ii)	"	100	1.12	1.159	0.1
" (iii)	"	100	1.12	1.069	0.2
" (iv)	"	100	1.20	1.347	—
" (v)	"	100	1.57	1.766	—
" (vi)	"	100	1.69	1.156	0.3
Jochberg (i)	1300	100	0.08	0.929	—
" (ii)	1800	100	0.22	1.247	—
Gepatch-Haus (i)	1925	100	1.47	1.546	—
Zwieselstein (i)	2300	150	0.167	1.400	—

\* For over- or under-dispersion the coefficient of dispersion should be greater or less than  $1 \pm 0.286$  for 100 quadrats, or  $1 \pm 0.232$  for 150 quadrats.

*Distribution of Arnica montana.* From the data of Table II it is clear that in only two out of fourteen sample areas is the distribution of *A. montana* at random. In the others marked over-dispersion is evident from the coefficient of dispersion values. Nevertheless, the regression between the logarithm of percentage absence and mean density is linear (Fig. 2).

TABLE II. *Distribution of Arnica montana in Alpine Meadows*

Plot size: 10 × 10 m. Quadrat size: 24 × 24 cm.

Locality.	Altitude (metres).	Number of quadrats per plot.	Mean density, plants per quadrat.	Coefficient of dis- persion.*	$\chi^2$ test value of <i>P</i> .
Saint-Anton am Arlberg (iii)	1650	100	1.38	1.505	—
" " (iv)	1750	100	2.49	1.232	0.30
" " (vii)	1500	100	4.47	1.263	0.80
Kuhtai (iii)	2000	100	5.64	1.768	—
" (v)	"	100	1.93	3.959	—
" (vi)	"	100	0.30	2.660	—
" (vii)	"	100	1.65	1.731	—
" (viii)	"	100	1.78	2.333	—
" (ix)	"	100	1.82	3.257	—
" (x)	"	100	4.90	2.678	—
Jochberg (ii)	1800	100	0.86	2.420	—
" (iii)	1300	100	0.32	2.897	—
Gepatch-Haus (i)	1925	100	0.10	3.131	—
Zwieselstein (i)	2300	150	0.113	2.440	—

\* For over- or under-dispersion the coefficient of dispersion should be greater or less than  $1 \pm 0.286$  for 100 quadrats, or  $1 \pm 0.232$  for 150 quadrats.

*Distribution of Campanula barbata.* The distribution data of *C. barbata* in ten sample areas is given in Table III. In only three areas can the distribution be considered to be random; in the others the plants are over-dispersed and the greatest aggregation is found in a community near the Sulzenauhütte. From Fig. 3 it is evident that in spite of aggregation when the logarithm of percentage absence is plotted against the mean density the points fall very closely on a straight line.

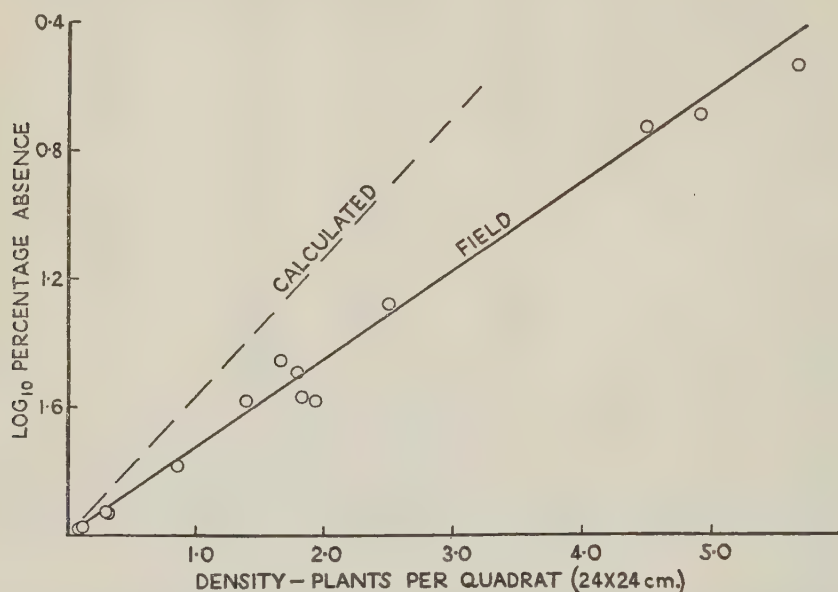


FIG. 2. *Arnica montana*. The relationship between the logarithm of percentage absence and mean density. The regression calculated on the basis of a random distribution is shown as a broken line.

TABLE III

*Distribution of Campanula barbata in Alpine Meadows*

Plot size: 10 × 10 m. Quadrat size: 24 × 24 cm.

Locality.	Altitude (metres).	Number of quadrats per plot.	Mean density, plants per quadrat.	Coefficient of dis- persion.*	$\chi^2$ test value of <i>P</i> .
Saint-Anton am Arlberg (i)	1600	100	0.06	1.287	—
" " (ii)	1650	100	0.10	1.113	—
" " (iii)	1700	100	1.26	1.405	—
" " (vii)	1500	100	0.36	1.264	0.02
Kuhtai (v)	2000	100	0.16	1.228	—
Jochberg (i)	1300	100	0.40	1.313	—
" (ii)	1800	100	0.24	1.357	—
" (iii)	1300	100	0.45	1.408	—
Sulzenauhütte (i)	1900	100	0.31	1.935	—
Obergurgl (i)	2100	200	0.755	1.497	—

\* For over- or under-dispersion the coefficient of dispersion should be greater or less than  $1 \pm 0.286$  for 100 quadrats or  $1 \pm 0.202$  for 200 quadrats.

*Distribution of Species in Chalk Downland.*

*Distribution of Senecio campestris.* For this particular species a number of sample areas scattered over the 'Seven Sisters', near Birling Gap, Sussex, was examined. In twelve out of fifteen plots the coefficient of dispersion is not significantly different from unity (see Table IV) and the distribution is

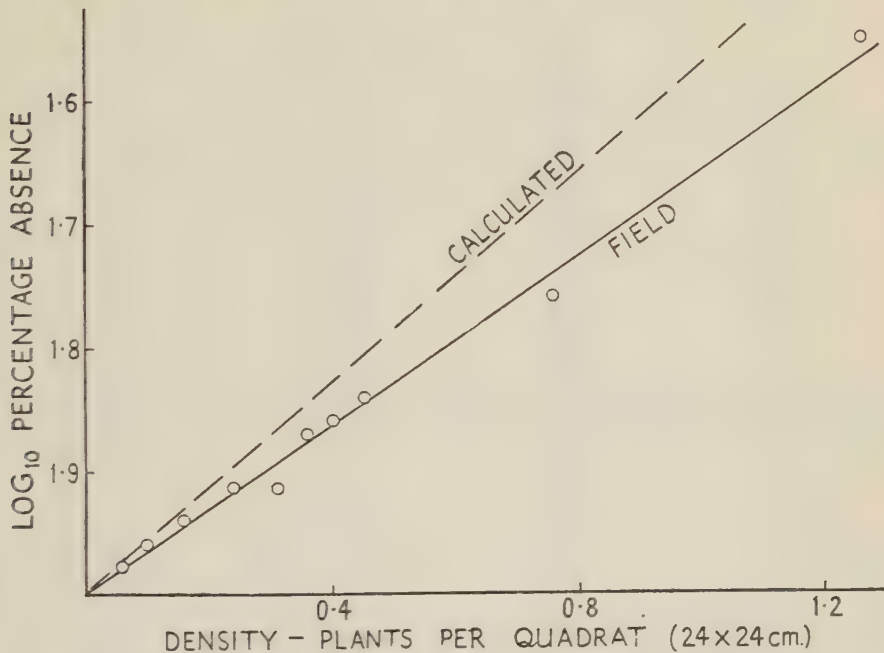


FIG. 3. *Campanula barbata*. The relationship between the logarithm of percentage absence and mean density. The regression calculated on the basis of a random distribution is shown as a broken line.

therefore at random. In six plots where the data allow of the  $\chi^2$  test this random distribution is confirmed. The regression of the logarithm of percentage absence against mean density is seen in Fig. 4. It is to be noted that at the higher densities the linear relationship is less good. This is to be expected on statistical grounds since it has been shown in the previous paper (Blackman, 1935) that when the percentage absence value falls below 20 per cent., i.e.  $\log 1.303$ , the error of the percentage absence estimate increases rapidly.

*Distribution of Cirsium arvense.* The distribution of *C. arvense* was investigated in a narrow block of thirty plots (i.e. three plots wide and ten plots long), A 1-10, B 1-10, C 1-10. The site was on the Purbeck Hills near Steeple, Dorset, and there was some indication from the topography that the area had in the past been ploughland, though at the time of the investigation there was a complete but thin cover of the usual chalk flora. From Table V it is

TABLE IV

*Distribution of Senecio campestris in Chalk Downland*

Plot size: 10 × 10 m. Quadrat size: 24 × 24 cm. Number of quadrats per plot = 100.

Plot number.	Mean density, plants per quadrat.	Coefficient of dispersion.*	$\chi^2$ test value of <i>P</i> .
1	0.17	1.195	—
2	0.22	1.063	—
3	0.24	1.231	—
4	0.30	1.179	—
5	0.38	1.105	—
6	0.49	1.052	0.80
7	0.60	1.784	—
8	0.73	0.965	0.90
9	0.88	1.247	0.05
10	0.97	1.300	—
11	1.03	1.137	0.50
12	1.69	1.264	0.30
13	2.47	0.764	0.05
14	2.87	1.483	—
15	4.04	3.061	—

\* For over- or under-dispersion the coefficient of dispersion should be greater or less than  $1 \pm 0.286$ .

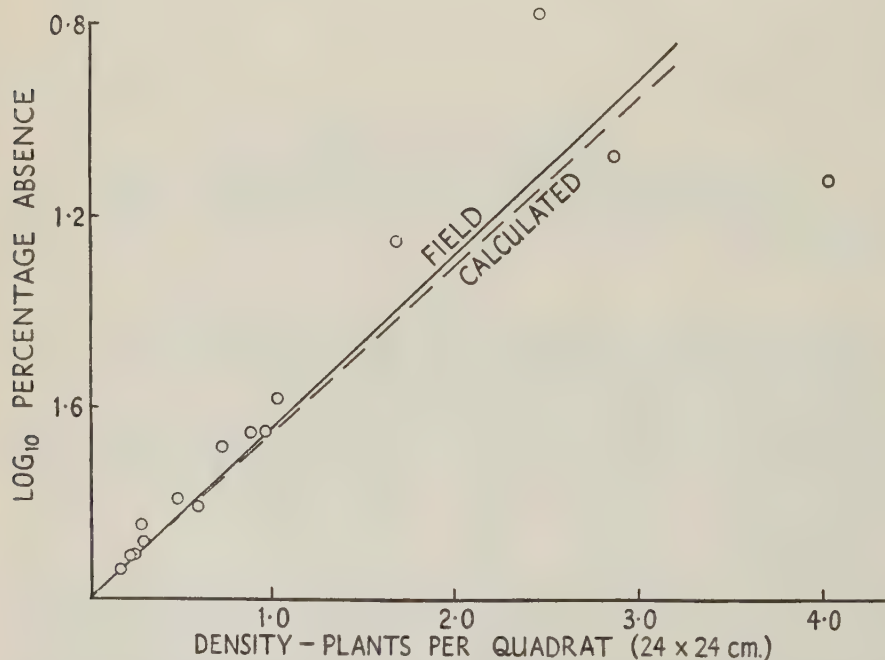


FIG. 4. *Senecio campestris*. The relationship between the logarithm of percentage absence and mean density. The regression calculated on the basis of a random distribution is shown as a broken line.



evident that, except in six plots, there is some degree of aggregation. Since there is a very considerable range in the coefficient of dispersion values (0.875 in plot C 1 as against 2.757 in plot B 7) it is not surprising that the relationship of mean density and the logarithm of percentage absence is only approximately linear (see Fig. 5).

TABLE V

*Distribution of Cirsium arvense in Chalk Downland*

Plot size: 30 × 30 ft. Quadrat size: 2 × 2 ft. Number of quadrats per plot = 100.

Plot and block number.	Mean density plants per quadrat.	Coefficient of dispersion.*	$\chi^2$ test. value of <i>P</i> .
A 1	0.12	1.057	—
2	0.42	1.212	0.01
3	0.19	1.048	—
4	0.73	1.934	—
5	0.46	1.424	—
6	0.47	1.524	—
7	0.50	1.474	—
8	0.97	1.444	—
9	0.70	1.198	0.3
10	0.92	1.311	—
B 1	1.01	1.949	—
2	0.70	1.631	—
3	0.45	1.364	—
4	0.66	1.477	—
5	0.75	1.545	—
6	1.28	2.161	—
7	1.40	2.757	—
8	0.19	1.244	—
9	0.81	1.415	—
10	1.10	1.699	—
C 1	0.87	0.875	0.30
2	0.70	1.631	—
3	1.00	1.697	—
4	0.87	1.525	—
5	0.80	1.995	—
6	0.41	1.237	0.10
7	0.61	1.288	—
8	1.06	2.474	—
9	0.81	1.788	—
10	1.07	1.440	—

\* For over- or under-dispersion the coefficient of dispersion should be greater or less than  $1 \pm 0.286$ .

*Distribution of Ophrys apifera.* A block of plots (4 × 3) was investigated on the north slope of Nine Barrows Down near Swanage in an area with an unusually high density of *O. apifera*. The relevant data are set out in Table VI. With the exception of plot 6 the plants were over-dispersed. The linear interdependence of the mean density and logarithm of percentage absence is seen in Fig. 6.

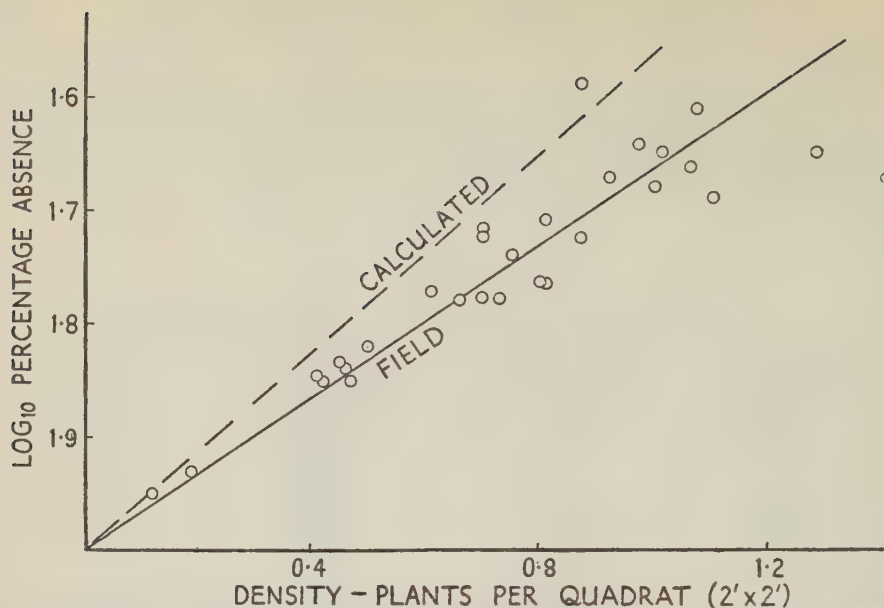


FIG. 5. *Cirsium arvense*. The relationship between the logarithm of percentage absence and mean density. The regression calculated on the basis of a random distribution is shown as a broken line.

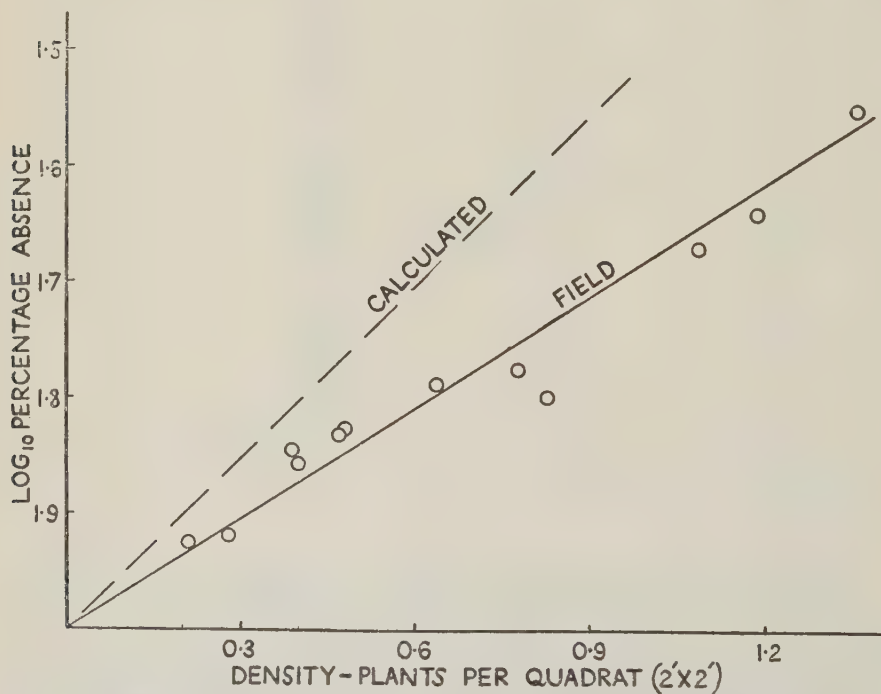


FIG. 6. *Ophrys apifera*. The relationship between the logarithm of percentage absence and mean density. The regression calculated on the basis of a random distribution is shown as a broken line.

TABLE VI

*The Distribution of Ophrys apifera on Chalk Downland*

Plot size: 25 × 25 ft. Quadrat size: 2 × 2 ft. Number of quadrats per plot = 100.

Plot and block number.	Mean density, plants per quadrat.	Coefficient of dispersion.*	$\chi^2$ test value of <i>P</i> .
A 1	1.36	1.686	—
2	0.83	2.411	—
3	0.28	1.689	—
4	0.21	1.663	—
B 1	0.78	2.661	—
2	0.47	1.481	—
3	0.39	1.134	0.20
4	1.09	1.670	—
C 1	0.48	1.536	—
2	0.40	1.363	—
3	1.19	1.710	—
4	0.64	1.658	—

\* For over- or under-dispersion the coefficient of dispersion should be greater or less than  $1 \pm 0.286$ .

*Distribution of Ranunculus bulbosus.* For *R. bulbosus* and the subsequent species the area investigated was the eastern end of Ballard Down near Swanage. This area differs from the other localities in that the chalk is nearer the surface, there is an abundance of flints, and the down is very wind-swept. The surface cover of vegetation is thin and individual plants very dwarf.

For data on the distribution of *R. bulbosus* a block of twenty plots ( $5 \times 4$ ) was examined. The results are seen in Table VII. It is clear that in every plot the plants were aggregated, since the coefficient of dispersion in each case significantly exceeds unity. The linear relationship between mean density and the logarithm of percentage absence is shown in Fig. 7.

TABLE VII

*The Distribution of Ranunculus bulbosus on Chalk Downland*

Plot size: 18 × 15 ft. Quadrat size: 10 × 10 in. Number of quadrats per plot = 100

Plot and block number.	Mean density, plants per quadrat.	Coefficient* of dispersion.	Plot and block number.	Mean density, plants per quadrat.	Coefficient* of dispersion.
A 1	1.97	2.465	C 1	2.08	2.959
2	1.41	3.842	2	2.37	2.802
3	1.76	2.240	3	1.42	2.166
4	1.77	2.441	4	1.50	2.431
5	1.72	2.551	5	0.27	2.608
B 1	1.37	2.266	D 1	1.51	3.391
2	1.16	1.667	2	0.22	2.349
3	1.57	2.087	3	0.65	2.902
4	2.05	2.704	4	0.59	4.181
5	1.90	2.749	5	0.85	1.928

\* For over-dispersion the coefficient of dispersion should exceed 1.286.

*Distribution of Gentiana Amarella.* The distribution of *G. Amarella* was investigated in two areas on Ballard Down. In one locality a block of twelve plots ( $3 \times 4$ ) was examined and in the other, where the average density was considerably lower, counts were made on six plots ( $3 \times 2$ ). The results are shown in Table VIII. It is seen that in both blocks, i.e. A 1-D 3 and

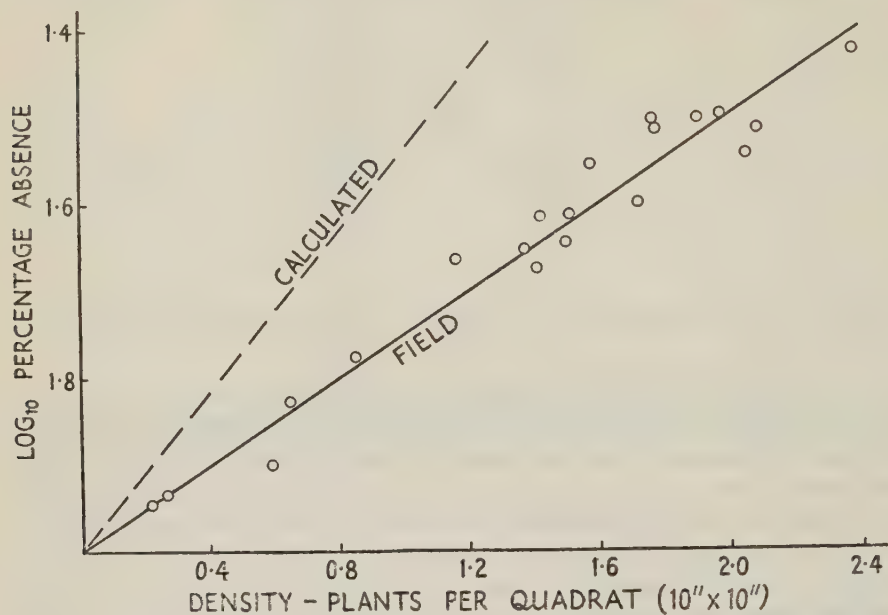


FIG. 7. *Ranunculus bulbosus*. The relationship between the logarithm of percentage absence and mean density. The regression calculated on the basis of a random distribution is shown as a broken line.

TABLE VIII

*Distribution of Gentiana Amarella on Chalk Downland*

Plot size:  $18 \times 15$  ft. Quadrat size:  $5.5 \times 5.5$  in. Number of quadrats per plot = 100.

Plot and block number.	Mean density, plants per quadrat.	Coefficient* of dispersion.	Plot and block number.	Mean density, plants per quadrat.	Coefficient* of dispersion.
A 1	3.13	2.608	D 1	2.25	2.814
2	1.97	2.281	2	1.94	1.247
3	1.60	2.102	3	3.09	1.793
B 1	1.20	1.885	E 1	0.45	1.544
2	1.82	1.736	2	0.40	1.516
3	1.31	2.726	3	0.76	4.017
C 1	0.51	1.486	F 1	0.83	2.265
2	0.77	1.334	2	1.60	2.815
3	1.29	1.865	3	1.24	1.843

\* For over-dispersion the coefficient of dispersion should exceed 1.286.



E 1-F 3, the plants are significantly over-dispersed except in plot D 2, where the distribution is random ( $\chi^2$  test  $P = 0.30$ ). The relationship between the logarithm of percentage absence and mean density is approximately linear (Fig. 8).

*Distribution of Erythraea Centaurium.* In the case of *E. Centaurium* the

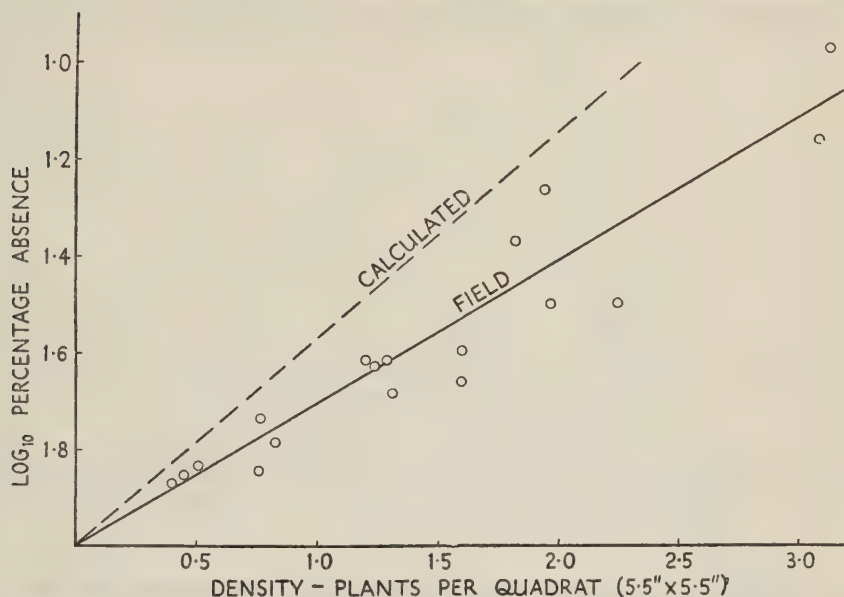


FIG. 8. *Gentiana Amarella*. The relationship between the logarithm of percentage absence and mean density. The regression calculated on the basis of a random distribution is shown as a broken line.

TABLE IX

*The Distribution of Erythraea Centaurium on Chalk Downland*

Plot size: 45 × 45 ft. Quadrat size: 3 × 3 ft. Number of quadrats per plot = 100.

Plot number.	Mean density, plants per quadrat.	Coefficient of dispersion.	Plot number.	Mean density, plants per quadrat.	Coefficient of dispersion.
1	1.94	5.07	7	3.22	13.41
2	2.52	10.23	8	4.42	7.34
3	2.33	10.78	9	1.92	10.99
4	0.50	4.34	10	2.79	3.95
5	3.14	10.54	11	3.65	9.08
6	0.21	2.14	12	2.40	13.40

sample plots were not grouped together but scattered over Ballard Down, since, except in patches, this plant was not present in the sward. It is therefore not unexpected to find that even within the sample areas this species is markedly over-dispersed. The values for the coefficient of dispersion given in Table IX are on average much in excess of those found for the previous

species, the highest figure being 13.41 in plot 7, whereas in Tables I–VIII the maximum value was 4.017 (Table VIII, plot E 3). From Fig. 9 it is evident that in spite of marked aggregation the relationship between mean density and the logarithm of percentage absence is approximately linear.

*Distribution of Poterium Sanguisorba.* For the distribution of this species

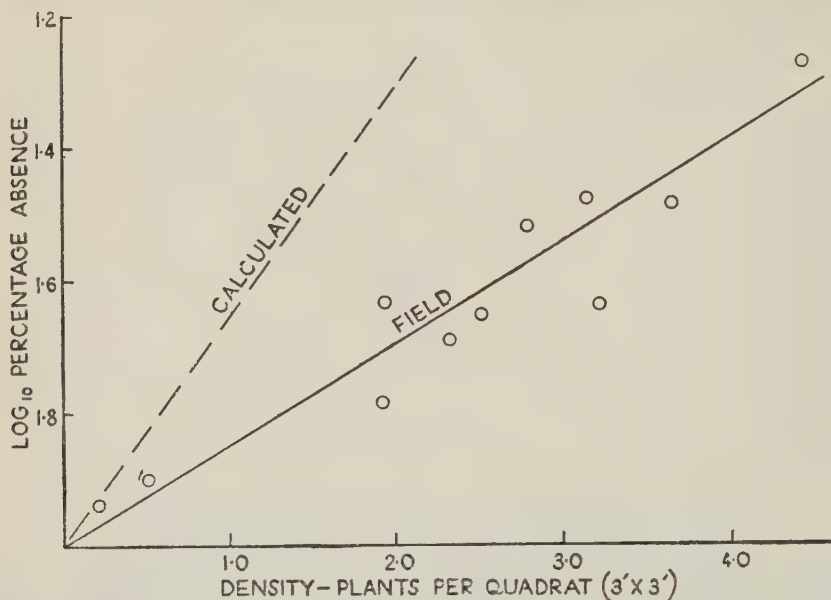


FIG. 9. *Erythraea Centaurium*. The relationship between the logarithm of percentage absence and mean density. The regression calculated on the basis of a random distribution is shown as a broken line.

a long, narrow block of plots ( $2 \times 10$ ) on Ballard Down was examined. From the data given in Table X it is clear that in fifteen sample areas the distribution was at random. Of the remaining five in only one was there any marked over-dispersion (A 5), while in one plot the plants were significantly under-dispersed (A 3). Thus it would be expected that the relation between mean density and the logarithm of percentage absence would be linear, and, moreover, that the regression should be in good agreement with that expected on the basis of a random distribution in all the sample areas. That this is so is seen in Fig. 10.

#### DISCUSSION

The results of this investigation show that in the species examined the distribution ranges from a random scatter to a marked aggregation of individuals. Only in the case of *P. Sanguisorba* is the distribution at random, although *S. campestris* is distributed by chance in the majority of the sites

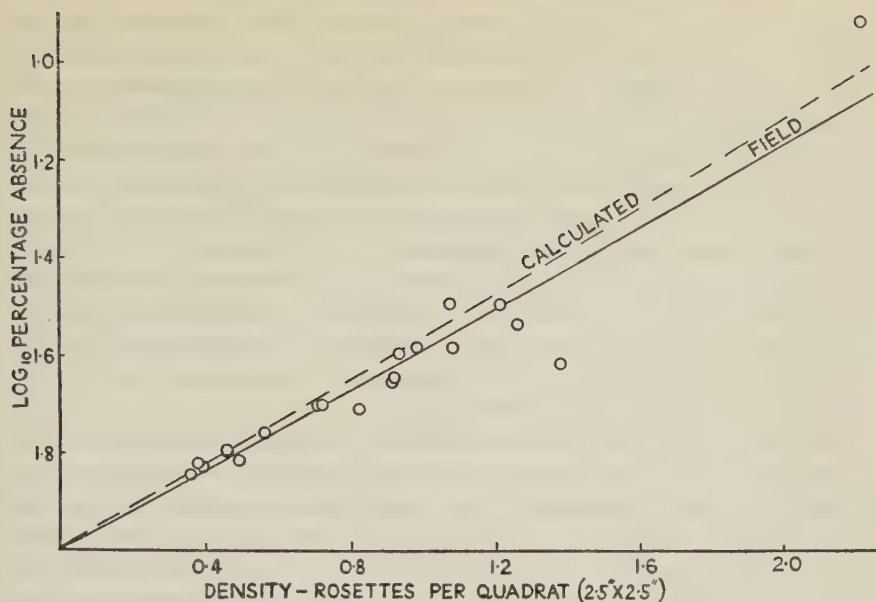


FIG. 10. *Poterium Sanguisorba*. The relationship between the logarithm of percentage absence and mean density. The regression calculated on the basis of a random distribution is shown as a broken line.

TABLE X

*The Distribution of Poterium Sanguisorba in Chalk Downland*

Plot size: 18 × 15 ft. Quadrat size: 2.5 × 2.5 in. Number of quadrats per plot = 100.

Plot and block number.	Mean density, plants per quadrat.	Coefficient of dispersion.*	$\chi^2$ test value of <i>P</i> .
A 1	1.07	0.930	0.10
2	0.98	0.927	0.70
3	2.21	0.621	—
4	1.21	0.990	0.30
5	1.38	1.798	—
6	0.92	1.058	0.20
7	0.46	0.941	0.50
8	1.08	0.948	0.02
9	0.72	0.984	0.30
10	0.49	1.175	0.05
B 1	0.56	0.914	0.30
2	0.36	1.208	0.20
3	1.26	1.261	0.20
4	0.91	1.023	0.02
5	0.93	0.918	0.50
6	0.38	0.839	0.20
7	0.39	0.927	0.80
8	0.82	1.143	0.01
9	0.46	0.897	0.80
10	0.71	0.976	0.20

\* For over- or under-dispersion the coefficient of dispersion should be greater or less than  $1 \pm 0.286$ .

observed. The remaining eight species, however, show a varying degree of over-dispersion. These findings therefore are in agreement with those obtained for other grassland communities, namely that some species are distributed at random but others are not. On the basis of Steiger's (1930) data Clapham's (1936) results showed that only three (the coefficient of dispersion differs significantly from unity for *Psoralea floribunda*) out of the forty-four prairie species were distributed at random. This lack of random distribution is more marked than that observed by Pidgeon and Ashby (1940) in their study, under Australian conditions, of regeneration following protection from grazing. They found that in twenty-four out of seventy-six sets of observations the distribution was at random. Their data are therefore more in line with the results of this investigation in regard to the proportion of species that are distributed at random.

It must, however, be pointed out that in the present investigation, sampling has been confined to small areas, while Steiger's relatively few quadrats were scattered over a considerable area which, from his account, showed a marked topographical and floristic variation. It is not therefore unexpected that a large number of species were not distributed at random and showed high coefficients of dispersion. On the other hand, where the heterogeneity of environmental conditions is low, such as might be expected in arable land, then the weeds invading bare fallow are mainly distributed at random according to the claims made by Singh and Chalam (1937) and Singh and Das (1938, 1939). The statistical interpretation of the data made by these workers must, however, be treated with very considerable reserve. In the first place, their mathematical calculations suffer from inaccuracies: for example, in Table I of Singh and Chalam's paper (1937) the coefficient of dispersion (relative variance) for *Ipomoea hispida* is incorrect, while in Table I of the second paper (Singh and Das, 1938) the mean density for *Gynandropsis pentaphylla* and the coefficient of dispersion for *Bonnaya brachiata*, *G. pentaphylla*, and *Ammania brachiata* (*A. baccifera*?) are wrongly calculated. In the second place, they have improperly applied the  $\chi^2$  test owing to their failure (i) to group together the frequency classes with values of less than five, (ii) to subtract *two* degrees of freedom from the total degrees of freedom before entering the  $\chi^2$  test (see p. 354 of this paper). In the third place, they have not realized that small departures from unity in the coefficient of dispersion cannot be taken to indicate a non-random distribution unless they significantly exceed the standard error. In the fourth place, the curves given in Fig. 4 of the third paper (Singh and Das, 1939), relating the size of the sampling quadrat to the mean number of species found in the quadrat, in no way agree, as they must do, with those calculated from the relevant data contained in Table II on the basis of the formula, correctly given on page 72. Nevertheless, when statistical tests are correctly applied to their data, it would appear that three-quarters of the species are distributed at random.

In consequence of this marked tendency in grassland communities for the



majority of species to be over-dispersed, an accurate measure of the mean density from a *single* set of observations cannot be calculated on the criterion of the proportion of quadrats containing no individuals of the species (Blackman, 1935; Clapham, 1936; Pidgeon and Ashby, 1940). It does not, however, follow that where species are over-dispersed changes in density cannot be assessed from changes in the percentage absence. As long as the relationship between the logarithm of percentage absence and mean density is known then fluctuations in density can be measured by variations in the percentage absence. In a previous paper (Blackman, 1935) it was shown that in spite of the aggregation of individual rosettes of *Plantago media* the relationship between the logarithm of percentage absence and density was yet linear. Similarly, in the present investigation it is clear from Figs. 1-10 that this relationship is approximately linear for all the species observed, irrespective of whether they are distributed at random or over-dispersed.

While therefore on the basis of this evidence changes in density are directly proportional to changes in the logarithm of percentage absence, the absolute changes in density cannot be determined without applying a correction factor where individuals are not distributed at random. Where species are over-dispersed it is found that for any given density the number of quadrats observed to contain no individuals is *greater* than would be expected if the distribution was at random (e.g. Figs. 2, 8, 9). In consequence for species which are over-dispersed the density estimate obtained from the equation

$$\log \frac{\% \text{ absence}}{100} = -m \log e$$

is too small, and in order to obtain the true density a correction factor  $K$  must be applied to the density estimate. Under conditions of aggregation the value of  $K$  must exceed unity, but if plants are under-dispersed the value of  $K$  may be less than one.

For the species observed in the present investigation the mean correction factor has been calculated from the results given in Figs. 1-10, and the values of  $K$  so obtained are shown in Table XI together with the mean coefficients of dispersion. It is to be noted that  $K$  is not directly proportional to the degree of over-dispersion.

On the basis of the evidence so far considered the results confirm the conclusion, reached in the previous paper (Blackman, 1935), that the estimation of percentage absence has many advantages for a study in the field of changes in the composition of communities where small areas are being studied in detail. That such estimations can be made quickly and lack personal bias has already been stressed. Moreover, the results of this investigation have shown that even when the distribution of species is not at random the relative changes in population can be expressed in terms of the logarithm of percentage absence; alternatively, the absolute changes can be measured if the individuals in the sampling quadrat are counted on a number of sites

and the correction factor determined. A small error may be introduced if the correction factor for low densities is applied to high densities, for it will be shown, in a subsequent paper, that for some species the density and coefficient of dispersion are correlated to some degree and so will modify to a small extent the value of the factor for different densities.

It must, however, be pointed out that unless *individual* plants can be identi-

TABLE XI

*The Relationship between the Coefficient of Dispersion and the Correction Factor K for the Estimation of Densities from the Logarithm of Percentage Absence*

Species.	Coefficient of dispersion mean value.	Correction factor K.
<i>Poterium Sanguisorba</i> . . .	1.024	1.07
<i>Senecio campestris</i> . . .	1.322	0.96
<i>Gentiana acaulis</i> . . .	1.329	1.20
<i>Campanula barbata</i> . . .	1.381	1.25
<i>Cirsium arvense</i> . . .	1.561	1.30
<i>Ophrys apifera</i> . . .	1.722	1.57
<i>Gentiana Amarella</i> . . .	2.104	1.47
<i>Arnica montana</i> . . .	2.312	1.64
<i>Ranunculus bulbosus</i> . . .	2.636	1.86
<i>Erythraea Centaurium</i> . . .	8.440	2.28

fied the correction factor cannot be determined, and in most closed grassland communities such identification is not possible for many of the species. With these species therefore there must be some uncertainty as to the interpretation of percentage absence data in terms of density. In the present study all the plants have been chosen because of their ready identification as individuals or entities. Several species were rosette plants (*A. montana*, *C. barbata*, *S. campestris*, *R. bulbosus*), in others (*G. acaulis*, *G. Amarella*, *E. Centaurium*, *O. apifera*) individuals were easily recognized. In the case of *P. Sanguisorba* individuals were best identified by their tap-roots, while the data for *C. arvense* were based on counts of the aerial shoots which may or may not have been single plants.

Nevertheless, in spite of the varying criteria used to estimate density there is a similar relationship between density and percentage absence. It is not therefore unreasonable to suppose that this relationship holds even in the extreme case of a stoloniferous plant, and in support of this it has been shown for *Trifolium repens* (Blackman, 1935) that percentage absence is a good measure of density where density has been expressed in terms of percentage area covered. If, however, this assumption is not accepted, then the alternative methods of estimating density—weight of shoots, tiller counts, area covered—are all far more laborious and in the case of tiller counts and area covered are subject to personal bias. Moreover, the statistical treatment of such data involves difficulties on account of their skew distribution curves (Morgan and Berulsdén, 1931; Blackman, 1935).

## SUMMARY

In a further study of plant populations the distribution of ten species in grassland communities has been investigated. In each sample area (100 square metres or less) the number of individuals in 100–200 random quadrats has been counted—in all 18,000 observations were made.

Data for *Arnica montana*, *Campanula barbata*, and *Gentiana acaulis* were collected in the Austrian Tyrol. The sample areas examined were not restricted to one locality, but were on the other hand confined to alpine meadows in which *Nardus stricta* was the dominant plant. For the remaining seven species—*Cirsium arvense*, *Erythraea Centaurium*, *Gentiana Amarella*, *Ophrys apifera*, *Poterium Sanguisorba*, *Senecio campestris*, and *Ranunculus bulbosus*—the observations were carried out on chalk downland in the south of England, and in the case of most species variations in distribution within a single community were studied by examining a block of plots.

In the statistical examination of the data it has been shown that departures from a random distribution can be tested by calculating the coefficient of dispersion, i.e. the variance divided by the mean. If the coefficient is greater or less than unity by  $2\sqrt{\left(\frac{2n}{(n-1)^2}\right)}$ , where  $n$  equals number of quadrats, then the plants are significantly over-dispersed or under-dispersed. If the coefficient of dispersion is not significantly different from unity, then the  $\chi^2$  test for the fitted Poisson distribution should be applied since the coefficient of dispersion is not a sensitive test for certain skew distributions.

The results have shown that the nature of the distribution is dependent upon the species and the locality. Only *P. Sanguisorba* can be considered to be distributed at random. Of the remaining nine species, since the coefficient of dispersion tends to exceed unity, the individual plants are over-dispersed, i.e. tend to be aggregated together in clumps. The degree of dispersion is least for *S. campestris*, *G. acaulis*, *C. barbata*, and *C. arvense*. With these four species the distribution may be at random in some areas and over-dispersed in others. For the other five species, a random distribution is rare or not found; the degree of over-dispersion is greatest for *A. montana*, *R. bulbosus*, and *E. Centaurium* (mean coefficients of dispersion 2.31, 2.63, and 8.44 respectively). In only one site was there any evidence of under-dispersion; here plants of *P. Sanguisorba* were markedly under-dispersed with a coefficient of dispersion of 0.62.

In spite, however, of the tendency of species to be over-dispersed it has been found that for each species the relationship between the logarithm of percentage absence (percentage number of quadrats containing no individuals) and mean plant density is approximately linear. Changes in density can therefore be estimated from changes in percentage absence even when the distribution is not at random. Under these conditions in order to determine the absolute changes in density from the percentage absence figures on



a logarithmic scale it is necessary to apply a correction factor. For the ten species this has been found to vary from 1.07 for *P. Sanguisorba* to 2.28 for *E. Centaurium*.

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# An Ecological and Taxonomic Study of the Algae of British Soils

## II. Consideration of the Species observed

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With eight Figures in the Text

## I. CHLOROPHYCEAE

### VOLVOCALES

OF the nine species of *Chlamydomonas* found, only three have been previously recorded, viz. *C. gloeocystiformis* Dill (Fig. 1, D) which formed small palmelloid colonies in moist cultures of soil 6, *C. platyrhyncha* Korschik. (Pascher, 1927, p. 271) in the same soil, and *C. minutissima* Korschik. in soil 11; the last two are new records for this country. Some of the individuals of *C. platyrhyncha* were curved, while those of *C. minutissima* showed a minute papilla on staining with methylene blue. The new species are as follows:

*Chlamydomonas subangulosa* n. sp. (Fig. 1, c). Cells broadly ellipsoidal, both ends rounded, the anterior with a broad flat papilla. Membrane thin. Chloroplast cup-shaped, faintly lobed in the older cells, the basal portion with the large oval, occasionally angular, pyrenoid filling more than half the cell. Nucleus above the pyrenoid. Eye-spot small, near the anterior end. Flagella slightly shorter than the body. Division usually into 4, first division longitudinal. Palmella-stages with structureless mucilage. Cells 16–20  $\mu$  broad; 20–4  $\mu$  long.

This species, which is characteristic of acid soils (22–4), resembles *C. angulosa* Dill, but has a different papilla, much shorter flagella, a smaller eye-spot, and slightly larger cells.

*Chlamydomonas calcicola* n. sp. (Fig. 1, F–I). Cells oblong, flattened on one face (Fig. 1, I), both ends broadly rounded, the anterior with a flat rounded papilla. Membrane thin. Chloroplast cup-shaped, lining most of the wall, pyrenoid lateral, lying in a thickening of the chloroplast (Fig. 1, F). Nucleus near the posterior end. Eye-spot streak-like, anterior. Flagella slightly longer

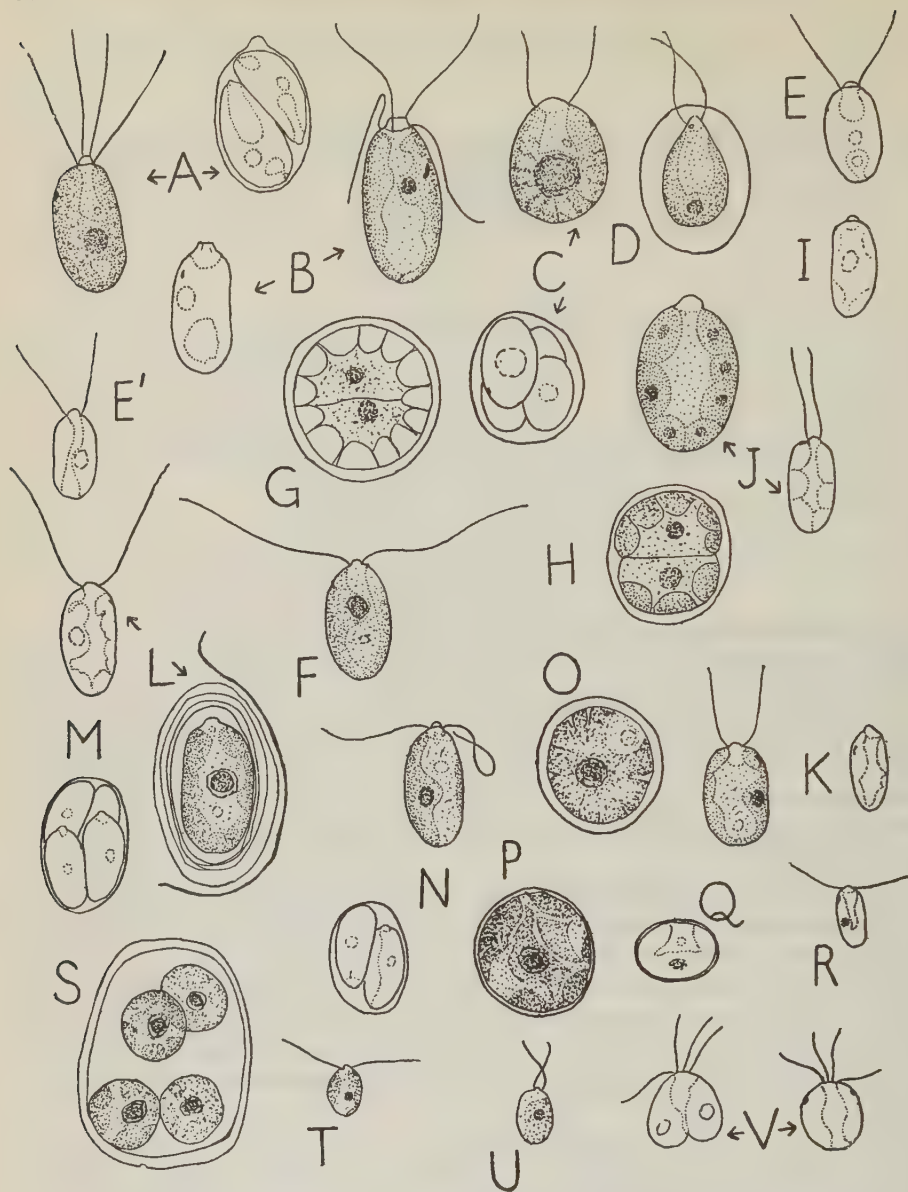


FIG. 1. A, *Carteria arenicola* n. sp., division-stage on the right. B, *C. acidicola* n. sp. C, *Chlamydomonas subangulosa* n. sp., division-stage below. D, *C. gloeocystiformis* Dill. E, E', *Chlamydomonas* sp. (soils 2, 12). F-I, *C. calcicola* n. sp.; G, H, stages in division of zygote; I, young individual. J, *C. Doonii* n. sp., below a young individual. K, *C. elliptica* Korschik. var. *britannica* nov. var. L, M, *C. pseudogloeogama* n. sp.; lower figure in L shows a cell with its mucilage-envelope; M, division-stage. N, *C. pseudo-elegans* n. sp. O-V, *Chlorococcum lobatum* (Korschik.) nov. comb.; R, T, zoospores; S, palmelloid stage; U, gamete; V, stages in sexual fusion. ( $\times 1,000$ .)

than the cell. Division into 2 or 4, the first division longitudinal. Zygotes (gamete-formation not observed) with a thick, smooth membrane (Fig. 1, G), sometimes surrounded by mucilage, the cell-contents generally obscured by peripheral white globules (fat ?). Division of contents (Fig. 1, G, H) into 4 or 8 flagellate cells observed on agar. Cells 10  $\mu$  broad; 14–16  $\mu$  long.

This species, usually observed in liquid cultures, grows well only on calcareous soils (3, 5, 7, 14, 20, 23, 24). It resembles *C. sphagnophila* Pascher (1930, p. 125), but differs in the presence of a papilla, in the shorter flagella, and in the streak-like eye-spot which is not raised.

*Chlamydomonas elliptica* Korschik. var. *britannica* nov. var. (Fig. 1, K). This variety differs from the type in having a flat, not sharply demarcated, papilla, in the flagella being as long as the body, and in the dimensions of the cells (7–10  $\mu$  broad; 12–14  $\mu$  long). The cup-shaped chloroplast reaches to the anterior end and is irregularly thickened, the lateral pyrenoid being situated in a median thickening. The eye-spot is curved. This variety occurred in liquid cultures of soil 13.

*Chlamydomonas pseudogloeogama* n. sp. (Fig. 1, L, M). Cells oval to sub-cylindrical, mostly slightly curved, both ends rounded, with a broad round papilla. Cell-wall thin. Chloroplast cup-shaped, with a deeply lobed inner contour; pyrenoid lateral, in the middle of the cell. Eye-spot minute, anterior. Flagella longer than the body. Division into 4 (Fig. 1, M). Formed globular Palmella-stages with firm lamellated mucilage (Fig. 1, L) on soil 19. When transferred to water from the moist cultures, all the cells escaped in less than 5 minutes. Cells 10–15  $\mu$  broad; 19–21  $\mu$  long.

This species differs from *C. gloeogama* Korschik. (Pascher, 1927, p. 267) in the lobed inner contour of the chloroplast, the larger cells, and in the formation of Palmella-stages.

*Chlamydomonas pseudo-elegans* n. sp. (Fig. 1, N). Cells oblong to almost cylindrical, sometimes with one face more flattened than the other, or even slightly curved, with a small pointed papilla. Cell-wall thin. Chloroplast lining the greater part of the wall, pyrenoid lateral. Nucleus just above the pyrenoid. Eye-spot streak-like, anterior. Flagella as long or shorter than the body. Division into 2 or 4. Cells 6–10  $\mu$  broad; 16–20  $\mu$  long.

This species, found in soil 11, resembles *C. elegans* West (Pascher, 1927, p. 279) in shape and chloroplast, but differs in the possession of a papilla and eye-spot, in the shorter flagella, and in the smaller dimensions of the cells.

*Chlamydomonas Doonii* n. sp. (Fig. 1, J). Cells broadly ellipsoid or ovoid, with a flat papilla. Cell-wall thin. Chloroplast as a whole cup-shaped, divided into a number of sections, each with a pyrenoid. Eye-spot small, anterior. Flagella longer than the cell. Cells 10–16  $\mu$  broad; 20–4  $\mu$  long.

This species, found in soil 16, resembles *C. apiculata* Pascher (1927, p. 289), but differs in the shape of the papilla, in the extension of the chloroplast to the anterior end, and in the possession of an eye-spot. From *C. subreticulata*

Pascher (1932, p. 47) it differs in the less broad rounding of the ends, in all the pyrenoids being of equal size, and in the position of the eye-spot.

The small *Chlamydomonas* shown in Fig. 1, E, E' is possibly also new; it is distinguished by the chloroplast which leaves both ends free and has the pyrenoid within a marked thickening, a little to one side.

*Carteria arenicola* n. sp. (Fig. 1, A). Cells ellipsoidal, somewhat curved, both ends broadly rounded, with a rounded papilla. Membrane thin. Chloroplast in optical section approximately H-shaped, the two limbs of the H incurved at the posterior end, the single pyrenoid situated a little to one side in the cross piece near the middle of the cell. Nucleus anterior. Stigma small, lens-shaped, in the anterior third of the cell. Flagella coarse, longer than the body. Division usually into 2. Cells 10–14  $\mu$  broad; 18–20  $\mu$  long.

This was found in soils 1 and 9 and resembles *C. micronucleolata* Korschik. (Pascher, 1927, p. 156), but differs in its curved cells, in the shape of the papilla, in the detailed structure of the chloroplast, and in the position of the nucleus.

*Carteria acidicola* n. sp. (Fig. 1, B). Cells cylindrical to narrowly ellipsoidal, slightly curved, the anterior end usually broader than the posterior and with a broad truncate, apparently cruciform papilla, from the four edges of which the flagella arise. Cell-wall thin. Chloroplast a dark-green parietal plate, with lobed inner margin in the older cells, the lateral pyrenoid in the middle of the cell. Nucleus in the posterior half. Stigma elongate, in the anterior third of the cell. Flagella as long as the body. Division into 2–4, first division longitudinal. Mature cells 12–16  $\mu$  broad; 18–24  $\mu$  long; young cells 10–12  $\mu$  broad; 16–19  $\mu$  long.

This species (soil 22) grows best in very dilute and acid solutions, with a pH of 3–4. It resembles *C. crucifera* Korschik. (Pascher, 1927, p. 157) in the papilla and the shape and size of the cell, but differs in the lack of striation in the chloroplast and in the lateral pyrenoid, as well as in the elongate eye-spot.

## CHLOROCOCCALES

### *Chlorococcum and Hypnomonas*

In all the soils investigated, except 13 and 14, where their occurrence is uncertain, *Chlorococcum*-like cells were present. Some, when grown on agar, showed two contractile vacuoles (Fig. 2, G), one of the distinguishing features of the genus *Hypnomonas* (Korschikoff, 1926), which is also characterized by the cells occurring singly or in palmelloid aggregates, by the chlorococcoid chloroplast, and by the presence of a cell-membrane around the swarms. This last feature and the presence of contractile vacuoles are thus the only differences from *Chlorococcum*. Korschikoff described two species from stagnant water, viz. *Hypnomonas chlorococcoides* with the general structure of *Chlorococcum humicolum* and *H. lobata* with a less dense, lobed chloroplast.



The *Chlorococcum*-cells from soils 2, 3, 7, 12, 18, 21, as well as one of the two forms present in 22 (referred to below as 22a), showed the presence of contractile vacuoles when grown on Benecke agar, about two weeks after the culture was started. Korschikoff transferred his material from agar to boiled

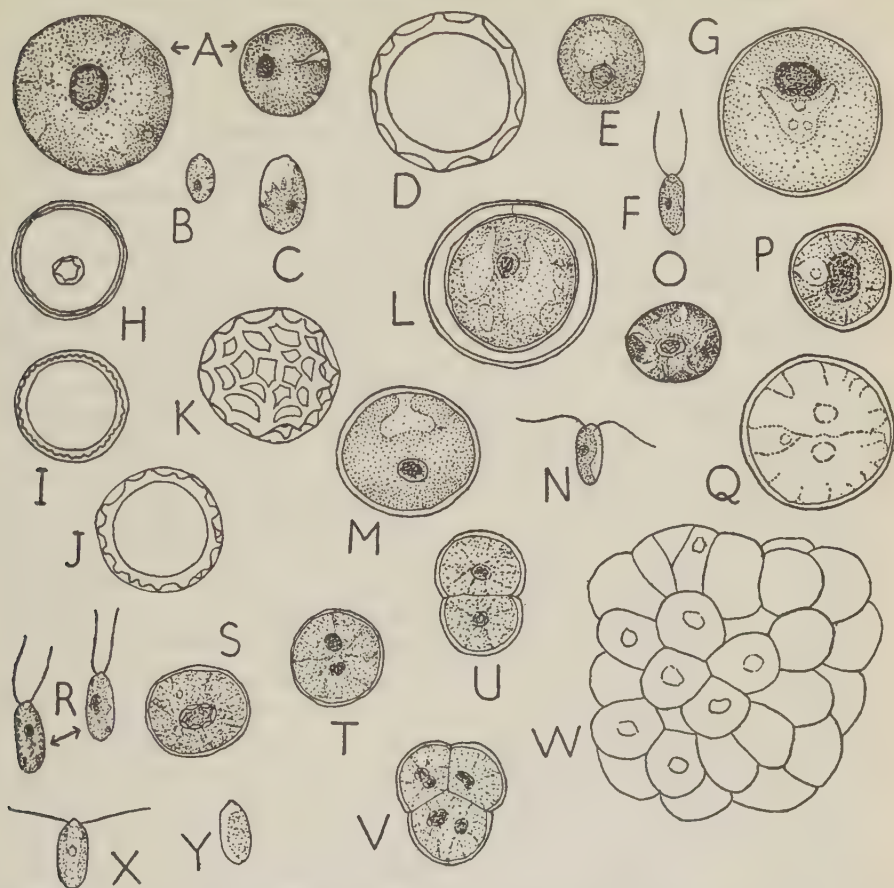


FIG. 2. A-D, *Chlorococcum lobatum* (Korschik.) nov. comb. var. *tenue* nov. var.; A, mature cells; B, C, zoospores; D, Trochiscia-stage. E-K, *C. humicolum* (Naeg.) Rabenh.; E, mature cell from soil 22; F, zoospore of same; G, mature cell from soil 21; H-K, stages in formation of Trochiscia-stage. L-N, *C. humicolum* var. *incrassata* nov. var.; L, old, and M, young cells; N, zoospore. O-R, *Macrochloris dissecta* Korschik.; O, young, and P, Q, older cells; R, zoospores. S-Y, *Borodinella polytetras* Miller; S, cell; T-V, division-stages; W, aggregate; X, Y, zoospores. (C  $\times 1,400$ ; the rest  $\times 900$ .)

water in order to obtain starch-free cells, but in this investigation this proved to be unnecessary. The *Chlorococcum* in soil 19 did not at first show contractile vacuoles, either on agar or in boiled water, but, when some of the soil from the original collection was again placed in Benecke solution, such vacuoles were detected. It would therefore appear that the development of contractile vacuoles, even in the same form, is variable and possibly depends

on the realization of unknown factors. The occurrence of contractile vacuoles in the *Chlorococcum* in soil 8 and in the second form present in soil 22 (referred to below as 22*b*) remains uncertain.

The appearance of contractile vacuoles in so many of the *Chlorococcum*-forms suggests that all those recorded from the soil may be capable of producing them under given conditions. In view of this, the maintenance of *Hypnomonas* as a separate genus becomes questionable, since the only difference lies in the presence or absence of a membrane around the zoospores. It was not possible to ascertain whether such a membrane was present in all the *Chlorococcum*-forms found, but in those from soils 12 and 21 and in 22*a* an extremely delicate membrane was demonstrated around the swarmer on plasmolysis with a strong solution of osmic acid. Previous workers have probably paid no special attention to this feature, but even should some of the forms here and elsewhere described possess naked zoospores, that fact alone would not justify the distinction of two genera. It is therefore proposed to merge *Hypnomonas* in *Chlorococcum*, the generic description of which must be amplified by the addition of: 'Cells with or without contractile vacuoles; zoospores often (always?) possessed of a delicate membrane.'

Associated with the *Chlorococcum*-cells were isolated cell-aggregates resembling *Trochiscia reticularis* (Reinsch) Hansg. (Fig. 2, D, J, K). Similar cells appeared in cultures, inoculated with a few *Chlorococcum*-cells, and aroused the suspicion that they represented a stage in the life-history of the latter. To test this, eight *Trochiscia*-cells were isolated, washed in distilled water, and transferred to an agar plate. Each of these cells gave rise to *Chlorococcum*-individuals. A few of these were transferred to a fresh agar plate and, after three months, *Trochiscia*-cells again appeared among the *Chlorococcum*-growth. Material both from soils 18 and 22 was tested in this way and gave identical results, though it was found that some of the *Trochiscia*-cells gave rise to similar cells for some time before *Chlorococcum*-individuals appeared. The *Trochiscia*-cells were commoner in Benecke solution than on agar. Since gametes were not observed in these particular forms, it is unlikely that these stages represent zygotes, and it seems more probable that they are resting-stages. This is further supported by the fact that the *Trochiscia*-cells are of all sizes, and that all gradations from a smooth wall to one with reticulate thickenings are found (Fig. 2, H-K).

The cells of the *Chlorococcum*-forms studied show great variation in size, thickness of wall, form of chloroplast, and size and shape of zoospore, and it is not improbable that a number of species should be distinguished. In some (e.g. from soil 21) the chloroplast was that typical of *C. humicolum* (Fig. 2, G), but in others it was pronouncedly lobed and more like that of *Hypnomonas lobata* (Fig. 2, A); all gradations were, however, found between the two types. It has not been thought advisable to establish new species, but for the benefit of subsequent workers the following forms are distinguished:

*Chlorococcum lobatum* (Korschik.) nov. comb. (Fig. 1, O-V). Cells spherical

or oval, single or in aggregates or in palmelloid expanses (Fig. 1, s). Membrane thick. Chloroplast not massive, markedly lobed, with a single pyrenoid. Two contractile vacuoles in the opening of the cup-shaped chloroplast. Trochiscia-stages rare. Zoospores (Fig. 1, r) with a thin membrane. Mature cells 19–22  $\mu$  broad; zoospores 5  $\mu$  broad, 8  $\mu$  long.

This is characteristic of alkaline and calcareous soils (2, 3, 7 (two forms), 12). One of the forms in soil 7 (Fig. 1, s–v) differed from the others in usually occurring in a palmelloid condition (Fig. 1, s), in having smaller zoospores (Fig. 1, t; 3.5  $\mu$  broad, 4.7  $\mu$  long), and in the formation of gametes (Fig. 1, u, v).

*C. lobatum* var. *tenue* nov. var. (Fig. 2, A–D). Differs from the type in its very thin-walled cells. Characteristic of acid soils (16, 18, 19, 22a).

*C. humicolum* Naeg. (Fig. 2, E–K). Characteristic of acid soils (21, 22b). The form in soil 22 lacked contractile vacuoles and was smaller (Fig. 2, E, F).

*C. humicolum* var. *incrassata* nov. var. (Fig. 2, L–N). Differs from the type in the thick (up to 8  $\mu$ ) and often lamellated walls. Soil 8, mostly in a palmelloid condition, no contractile vacuoles.

*Macrochloris dissecta* Korschikoff, 1926, p. 476; Printz, 1927, p. 451; Petersen, 1932a, p. 33 (Fig. 2, O–R). This alga was first described from bogs near Kharkov, Russia, being distinguished by its spherical cells with a chloroplast composed of a central piece containing one or more pyrenoids and several radiating processes (Fig. 2, P, Q) which spread out peripherally as discs or bands. They are stated to have several nuclei, but according to Petersen there is often only one, and all the British material was uninucleate. The biflagellate zoospores (Fig. 2, R) possess a membrane. Petersen found this alga in beech-wood and grassland soils in Denmark, and in this investigation it has also for the most part been found in grassland soils (2, 7, 15, 20, 23, 24).

The cells occur singly. Young ones (Fig. 2, o) are oval, while older ones (Fig. 2, P, Q) are globose and up to 42  $\mu$  in diameter. The rather thick membrane is composed of an outer firm and an inner gelatinous layer. The chloroplasts in the young cells have few radiating lobes, but in the older ones these subdivide into many small lobes. There is usually a single pyrenoid, but up to four may be found in the centre of the chloroplast. In certain older cells the latter divides into 2 or 4 segments, each with its own pyrenoid, as described by Petersen. The cells produce 2, 4, or 8 zoospores (Fig. 2, R) which are ellipsoidal, 4  $\mu$  broad and 12  $\mu$  long, with a thin membrane and a very small flat papilla visible only after staining; the chloroplast is irregularly lobed, with a single pyrenoid, and the eye-spot is anterior.

*Borodinella polytetras* Miller (1927) (Fig. 2, s–w). This genus is characterized by its large colonies (Fig. 2, w) formed of round cells which occur singly or in tetrahedral groups (Fig. 2, v), the cells possessing an axile chloroplast with a single pyrenoid. Multiplication is effected by division, as well as by zoospores. Miller found the alga, which has not since been recorded, in association with *Prasiola*.



In cultures of soil 22 this species formed a thick green scum on the surface of the liquid and on the sides of the culture bottles. The isolated cells (Fig. 2, s) are spherical, while the larger colonies (Fig. 2, w) are composed of tetrahedral groups (Fig. 2, v), which can be separated by pressure. The wall is thin and firm. The axile chloroplast has a central or subcentral pyrenoid with a well marked starch-sheath (Fig. 2, s), while the single nucleus lies towards one side of the cell. Cell-division takes place successively into four parts. The pyrenoid divides before the chloroplast (Fig. 2, t) and is to be observed throughout the process of division.

In the formation of zoospores the contents divide into 8 or 16 parts which escape by the rupture of the wall. The zoospores (Fig. 2, x, y) are usually cylindrical, but often slightly curved. They have a thin wall with a papilla at the anterior end, while the chloroplast is a curved parietal plate, containing a lateral pyrenoid. The flagella are longer than the body and there is a linear stigma near the anterior end. The zoospores measure 5–6  $\mu$  broad, 10–12  $\mu$  long.

*Muriella magna* n. sp. (Fig. 3, A–D). The genus *Muriella* was founded by Petersen (1932b, p. 402) to include unicellular Green Algae having many chloroplasts without pyrenoids or starch, and devoid of zoospores; in the last respect it differs from *Dictyococcus*. *Muriella terrestris*, on which the genus was based, was found in cultures of Danish soils. Vischer (1936, p. 403) has described two more species and amplified the generic description to include forms which contain oil and form starch when grown in cultures with sugar.

The alga here described, which was met with in a large number of soils (1, 6, 7, 9?, 13, 16, 18, 20–2, 24), has isolated cells (Fig. 3, A), up to 20  $\mu$  in diameter. They grow profusely when transferred to Benecke agar, forming rough dark green patches, containing cells reaching a diameter of 90  $\mu$ . The globular cells (Fig. 3, A) are occasionally protruded on one side. The wall is thick and brittle, being easily broken by pressure, and shows occasional lens-shaped, dark brown, thickenings which stain a deep purple with chlor-zinc iodide, while the rest of the wall only takes on a light violet tint. The younger cells (Fig. 3, B, C) contain from two to several parietal chloroplasts which are protruded inwards perpendicular to the wall; in surface view they appear polygonal or lens-shaped. In older cells the chloroplasts are numerous and closely packed (Fig. 3, A). Pyrenoids are absent, but starch occurs in small quantity. When the cells dry, large globules of orange-coloured oil appear within the contents. Daughter-cells, which are produced in large numbers, are set free by gelatinization of the wall on one side. The species is distinguished by its large cells and the numerous closely packed chloroplasts.

*Dictyosphaerium terrestre* n. sp. (Fig. 3, E–J). Cells spherical when mature, oval when young, up to 12  $\mu$  in diameter, disposed in groups of four surrounded by mucilage, the different groups connected by forked mucilage-



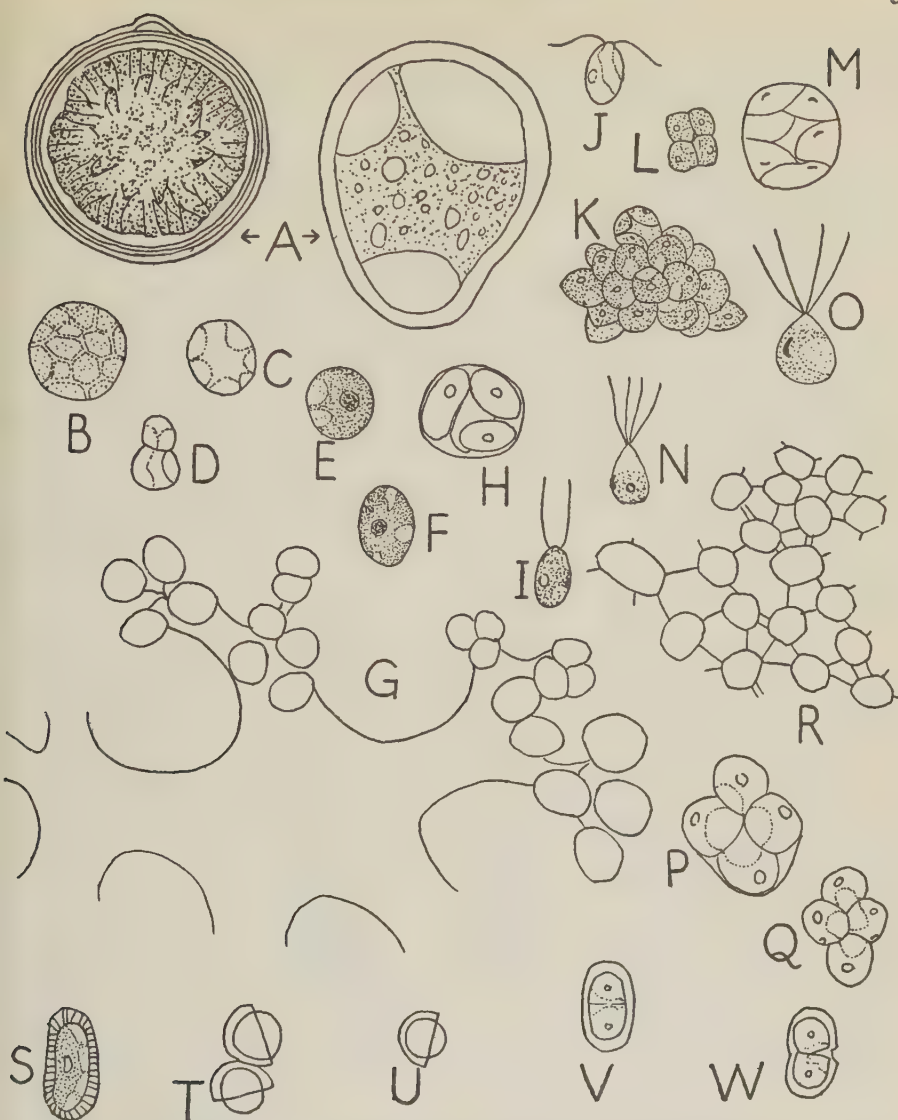


FIG. 3. A-D, *Muriella magna* n. sp.; A, old cells; B-D, young cells, in B stained with iodine and seen from the surface. E-J, *Dictyosphaerium terrestre* n. sp.; E, F, individual cells; G, part of a colony; H, formation of zoospores; I, J, zoospores. K-Q, *Fernandinella alpina* Chod. var. *emiglobosa* nov. var.; K, L, colonies; M, zoospore-formation; N, O, zoospores; P, Q, aplanospore-formation. R-W, *Interfilum paradoxum* Chod. & Topali var. *reticulatum* nov. var.; R, colony; S, individual cell stained with methylene blue; T-W, cell-division. ( $\times 800$ .)

strands (Fig. 3, G), as in *D. Ehrenbergianum* Naeg. Cell-wall thin, but on agar certain bigger cells (up to  $15\ \mu$ ) have thick walls. Chloroplast parietal, with two-many deep lobes and a single pyrenoid (Fig. 3, E, F). Multiplication by division into four. Zoospores (Fig. 3, I, J), which are commonly formed to the

number of four per cell (Fig. 3, H), are ellipsoidal, with a small papilla and flagella longer than the cell. The chloroplast is a parietal lobed plate with a pyrenoid, while the eye-spot is anterior.

This species was found in soils 7, 14, 20, 23, 24. A species of *Dictyosphaerium* (*D. minutum*) has previously been recorded from the soil by Petersen (1932a, p. 37).

*Fernandinella alpina* Chod. var. *semiglobosa* nov. var. (Fig. 3, K-Q). *F. alpina*, described by Chodat (1922) from cultures of alpine soils, is a colonial alga distinguished by the markedly pyriform cells with the pointed ends facing outwards, and by the formation of autospores which remain adhering to the 4-lobed cup into which the mother cell-membrane splits. The cells multiply like those of *Dictyosphaerium*, but the products do not become displaced radially, cohering to form dense syncoenobia. Petersen recorded an alga from soils of Iceland (1928b, p. 19), and Hammer Bakker (1932a, p. 34) which he identified with *F. alpina*, although the beak-like protuberance of the cells was less marked and the characteristic method of autospore-formation was not seen; he observed quadri-flagellate zoospores, which were also seen by Chodat, although the latter was unable to determine the number of flagella.

A very similar alga, found in liquid cultures of soils 3, 5, 7, 9, 14, and 20, occurred as single rounded cells or as 4-32-celled colonies (Fig. 3, K) in which the individual cells were often pointed at the end facing outwards, although a large number were completely spherical. There was profuse growth on agar, but here most of the cells were spherical and larger (up to 12  $\mu$  in diameter). Four-celled colonies (Fig. 3, L) resemble a *Crucigenia* having the pointed ends facing outwards. The cells are 8-10  $\mu$  in diameter and have a thin membrane. The parietal chloroplast, with a pyrenoid, occupies the outer face of the cell (Fig. 3, K) and leaves the inner part free.

During multiplication the daughter-cells sometimes possess eye-spots immediately after division, although they remain immotile. The characteristic splitting of the cell-wall described by Chodat was not observed, although the four daughter-cells remain attached to the empty membrane (Fig. 3, P). The zoospores, 1-8 of which are produced in a cell (Fig. 3, M), vary in size (Fig. 3, N, O); they are broadly rounded at the posterior and pointed at the anterior end, 5-7  $\mu$  broad and 11  $\mu$  long, with a prominent, linear, projecting eye-spot and four flagella which are longer than the body. The variety differs from the type in the larger and usually more rounded cells and in the details of daughter-cell formation.

Apart from the forms above considered, the following Chlorococcales were encountered in the soils examined: *Trebouxia arboricola* Puymaly (heath soils 17, 22); *Chlorochytrium paradoxum* (Klebs) West (cells oblong, up to 140  $\mu$  long, cf. Bristol, 1920, p. 21; only on chalk soils 4, 5, 7); *Chlorella vulgaris* Beij. (soils 6, 16-18, 21, 22); *Dactylococcus bicaudatus* A. Br. (mostly in non-calcareous soils, 7, 8, 13, 15, 18, 19, 22); *Coccomyxa dispar* Schmidle

(soils 6-8, 12, 13, 15-18, 21-3). In soils 21 and 22 there was an undetermined *Coccomyxa*, with straight or lunate cells,  $3-4\ \mu$  broad and up to  $30\ \mu$  long, pointed at both ends; chloroplast a parietal plate without pyrenoid.

## ULOTRICHALES

*Interfilum paradoxum* Chod. & Topali; Chodat, 1922 (Fig. 4, A-J). This curious alga was first found epiphytic in the mucilage of *Ophrydium versatile*

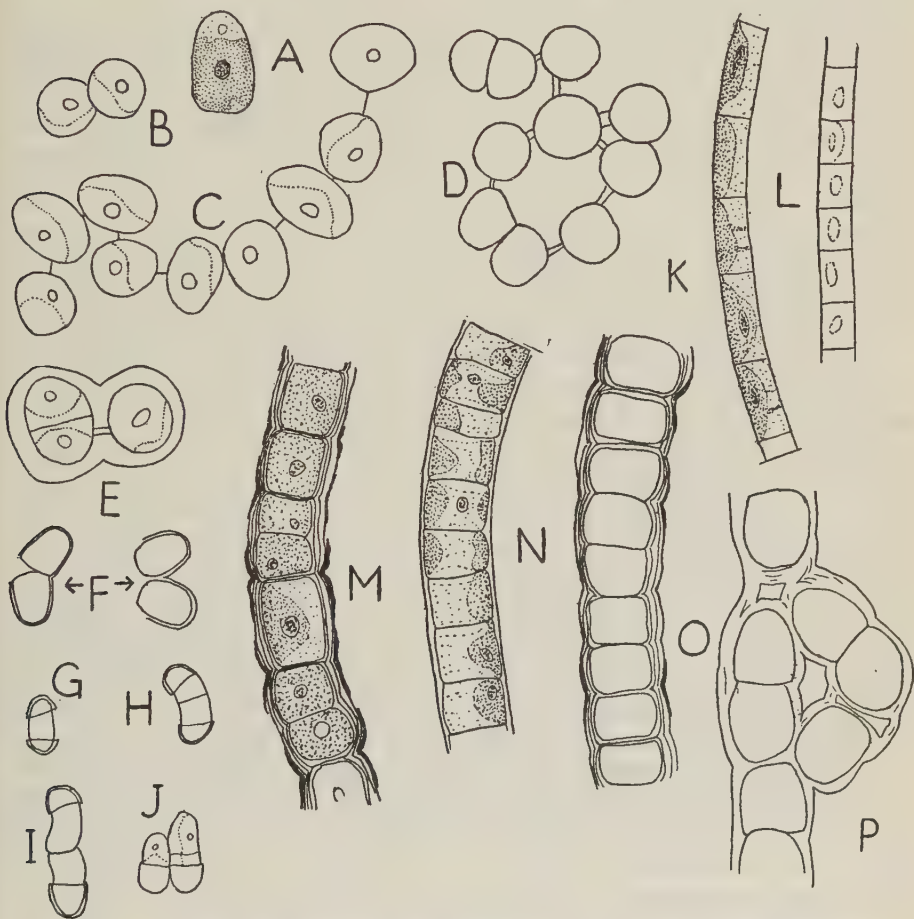


FIG. 4. A-J, *Interfilum paradoxum* Chod. & Topali; A, B, unstained cells; C, unstained filament; D, stained with methylene blue; E, cells mounted in Indian ink; F-J, cell-division. K-L, *Hormidium nitens* Menegh. M-P, *H. crenulatum* Kütz.; M, var. a; N-P, var. b. (A-E  $\times 1,300$ ; F-L  $\times 850$ ; M-P  $\times 450$ .)

near Geneva. It possesses spherical cells, occurring singly or in pairs or in simple or branched chains and provided with a bipartite membrane. The chloroplast is plate-shaped, with more or less sinuous edges and a single pyrenoid. On mounting in Indian ink a gelatinous investment becomes

apparent round each cell and shows radial striation on staining with methylene blue. The cells multiply by division along one plane, the products diverging first on one side so as to leave a triangular space between them, although subsequently separating completely. The daughter-cells are held together by a delicate thread, the origin of which was not established; branching of the chains was ascribed to displacement of the daughter-cells. The authors inclined to refer the alga to the Ulotrichales and to place it near *Radiofilum* and *Geminella*.

The material here described was obtained from liquid cultures of soils 21 and 22. The cells occurred singly or in pairs (Fig. 4, B) or very occasionally in short chains of four cells, connected by narrow mucilage-strands. On Benecke agar longer chains (Fig. 4, C), as well as small nets (Fig. 4, D) in which occasional cells were connected simultaneously to three or four others, were produced. Later the connecting strands either disappeared completely or were reduced to plugs of mucilage.

The individual cells are spherical or oblong, 4–6  $\mu$  broad and up to 12  $\mu$  long, usually with one end broader than the other (Fig. 4, A, C), the broader ends of adjacent cells facing one another. The mucilage-sheath (cf. Fig. 3, S; 4, E), only visible after treatment with methylene blue or mounting in Indian ink, was never as wide as figured by Chodat and Topali, while a faint radial striation was only occasionally seen. In cell-division only the protoplast divides, and a separate membrane is formed around each half. Thereupon the wall of the parent-cell splits at the place of division (Fig. 4, F–H), so that one daughter-cell is found inside each half of the original wall. The ridge, to which Chodat and Topali refer, is the broken edge of the parent-wall. In separating the two daughter-cells leave a triangular space between them (Fig. 4, F), and at the side corresponding to the apex of the triangle, where the daughter-cells are still in contact, the two halves of the parent-wall are often connected. As the cells separate further and enlarge, the intact part of the wall of the mother-cell remains as the connecting strand. When this is very short the daughter-cells come to lie parallel to each other.

Under certain circumstances the wall of the parent splits completely into two halves before the daughter-cells separate (Fig. 4, G) and the former then appear as caps at opposite ends of the enlarging cells (Fig. 4, H, I). When this happens, no connecting strand is formed. It has not been possible to establish how the nets are formed.

*Interfilum paradoxum* var. *reticulatum* nov. var. (Fig. 3, R–W). Cells resembling those of the alga above discussed, but rarely showing union into filaments, were found in the Breckland soils. As a general rule they formed an elaborate network (Fig. 3, R), in which each cell was connected to 3–5 others. The mucilage-sheath (Fig. 3, S) was much more pronounced and showed clear radial striation after staining with methylene blue. The cells divide like those of the type (Fig. 3, T–W). The mode of origin of the connecting strands is not clear. It may perhaps be doubted whether *Interfilum*



is a true filamentous alga; its correct place may be among the colonial Chlorococcales.

*Hormidium nitens* Menegh. emend. Klebs (Fig. 4, κ, L). In liquid cultures the long filaments form a silky growth at the surface and break up into short, 2-10-celled lengths which are often found at the bottom. In moist cultures only short threads are produced, while on agar the alga forms a thin sheet. The cells are 5.7-10  $\mu$  broad and vary from slightly shorter than broad to three times as long as broad. The parietal chloroplast has rounded edges and leaves about one-third of the wall free; the pyrenoid is often elongate (Fig. 4, κ). No zoospores were observed. This species occurs in all soils and was frequently found growing on the soil at the time of collection.

*H. crenulatum* Kütz.; Brand, 1913, p. 70; Petersen, 1915, p. 109. Var. *a* (Fig. 4, M). The elongate filaments, which are 10-20  $\mu$  broad with cells  $\frac{1}{2}$ -1 times as long, show no tendency to fragment. On moist soil the cell-wall is thick and stratified, with an uneven edge (Fig. 4, M), and occasionally shows the H-shaped thickenings described by Petersen, while on agar the wall is smooth and rather thin. The chloroplast has rounded edges, but owing to its dense character in some cells it appears axile; there is a distinct pyrenoid with a starch-sheath, although neither Petersen nor Brand found one. Division of the cells is very occasionally longitudinal. No reproductive stages were observed. This form is characteristic of alkaline soils (3, 13).

Var. *b* (Fig. 4, N-P). This form, found in a liquid culture from soil 22 and also occurring in 20, 23, and 24, produces very long filaments (up to 24  $\mu$  wide) on agar. They have thick, more or less lamellated walls (Fig. 4, O), sometimes with H-shaped thickenings. The cells are usually constricted at the cross-walls, as in *Ulothrix moniliformis* Kütz. and may be very short. The chloroplast is like that of var. *a*. Occasional longitudinal division results in the formation of loops (Fig. 4, P). Sometimes most or all the cells round off and form akinetes. Although resembling *Ulothrix moniliformis* in appearance, it is much wider and has a chloroplast (Fig. 4, N) of the Hormidium-type; it resembles *H. crenulatum* in the thick lamellated walls and in dimensions.

Two other Ulotrichales were observed, viz. *Stichococcus bacillaris* Naeg. (soils 6-8, 15, 18, 21, 22) and a form doubtfully identified as *Gloeotila proto-genita* Kütz. (soils 5, 14, 20).

#### CHAETOPHORALES

*Pleurastrum terrestre* n. sp. (Fig. 5, I-R). Material of *Pleurastrum* was found in cultures of soils 1-4, 7-10, 12, 17 (?), 21, and 22, the growth consisting of spherical cells up to 15  $\mu$  in diameter, occurring singly or in groups, and of short branched filaments. When transferred to agar, thalloid expanses (Fig. 5, I, J), in which the central cells are rounded or oval, while the periphery is usually occupied by few-celled filaments, are produced; the cells of the latter reach 22  $\mu$  wide and 40  $\mu$  long, although occasionally very narrow (5.6-10  $\mu$ ) and elongate, as in *P. paucicellulare* Vischer (1933, p. 18). More

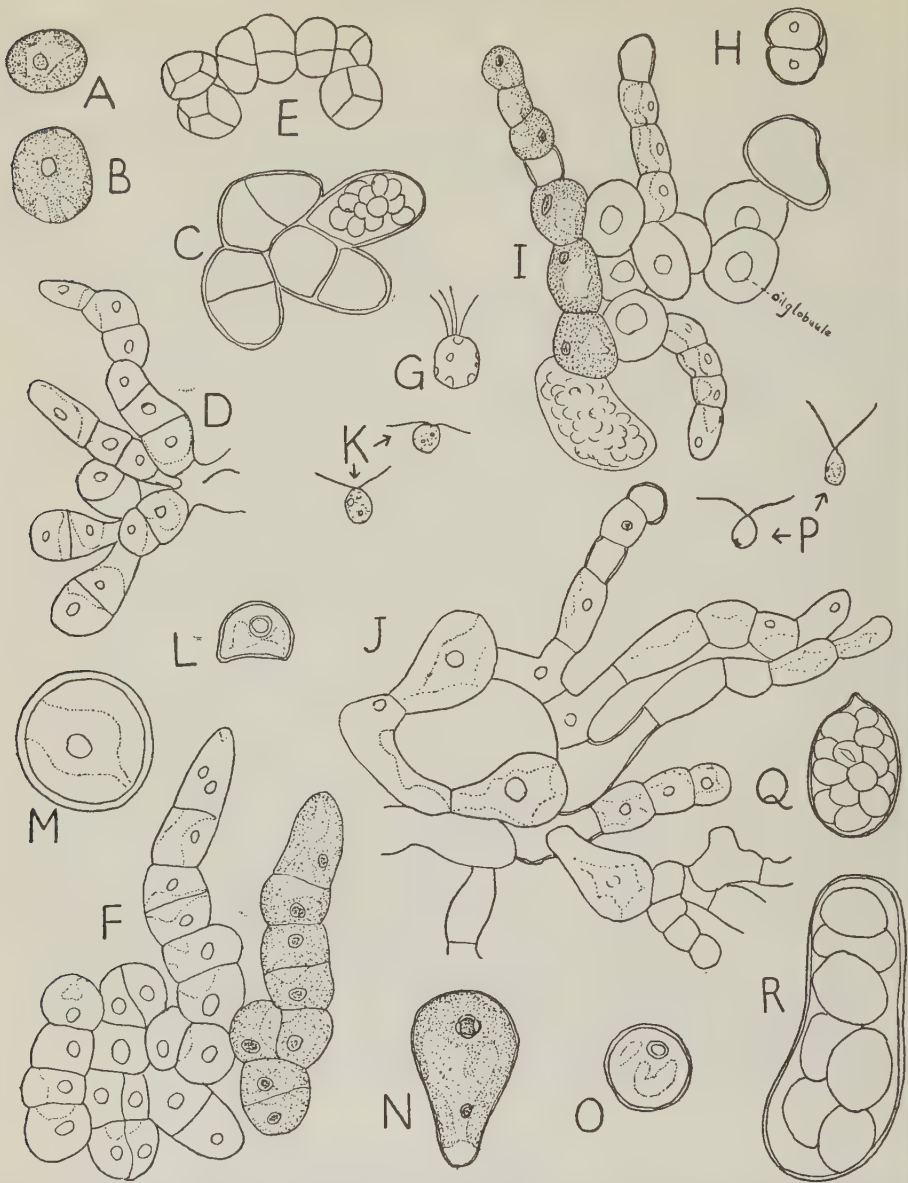


FIG. 5. A-H, *Pseudodoctonidium basiliense* Vischer var. *Brandii* Vischer; A, B, single cells; C-D, branched filaments from liquid cultures; E, F, the same from agar; G, zoospore; H, aplano-spores. I-K, *Pleurastrum terrestre* n. sp.; I, J, growth on agar; K, zoospores. L-R, *P. terrestre* n. sp., var. ?; L, M, single cells; N, O, cells from old cultures; P, zoospores; Q, R, aplano-spore-formation. (I, J  $\times 600$ ; the others  $\times 700$ .)

rarely compact pleurococcoid groups, composed of large cells resulting from division along three planes, are formed, these resembling the stages described by James (1936, p. 535) for *P. insigne* Chod. var. No palmelloid stages were observed.

The cell-wall, at first thin, becomes rather thick in older cells. The chloroplast (Fig. 5, I) lines a varying part of the wall, its edges being rounded and produced into lobes. Very little starch is present, but the older cells contain a large oil-globule which assumes an orange colour when the cells dry. Zoospores are formed in large numbers, both on agar and in the liquid cultures. Within the parent-cells they appear yellow-green and show a bright red eye-spot. When transferred to dilute culture solution or to tap water and exposed to bright light, they emerge through an opening in the wall, being at first enclosed in a vesicle. The free zoospores (Fig. 5, K) are ovoid in shape and slightly amoeboid, and usually contain a small oil-globule; the chloroplast is posterior, the eye-spot situated half-way down the cell, and the two flagella are longer than the body. Aplanospores (Fig. 5, Q, R), which are set free by the gelatinization of the cell-wall on one side, were also observed.

This species differs from *P. insigne* Chodat (Printz, 1927, p. 208) and *P. paucicellulare* Vischer (1933, p. 18) in its larger dimensions and the more numerous zoospores formed in a cell; from the former also in the elongate filaments and in the presence of an eye-spot. In general appearance and type of zoospore-formation it resembles *Leptosira obovata* Vischer (1933, p. 83), but the presence of one or more pyrenoids excludes reference to this genus. The absence of any previous record of this common soil alga may be due to the fact that its full characteristics can be clearly recognized only when grown on agar.

Single cells and two- or three-celled filaments which may belong to this species were found also in cultures of other soils, but could not be properly studied. Such stages also occurred in the soil from Kent's cavern, Torquay, being the only green alga present.

The *Pleurastrum* found in soils 21 and 22 differed in forming giant cells (Fig. 5, M, N), up to 40  $\mu$  broad and 70  $\mu$  long on agar, in having 1-4 pyrenoids in the cells, in the longer flagella of the zoospores (Fig. 5, P), and in its occurrence on acid soils; this is probably a separate variety. All the other soils in which *P. terrestre* occurred were alkaline.

*Pseudendoclonium basiliense* Vischer, 1926, p. 29 var. *Brandii* Vischer, 1933, p. 23 (Fig. 5, A-H).

The genus *Pseudendoclonium* of Wille (Printz, 1927, p. 208) is characterized by its rudimentary thallus with irregular branches and by the cells containing a single parietal chloroplast with a pyrenoid, while it differs from the closely related *Pleurastrum* in the presence of four flagella on the zoospores, and from *Stigeoclonium* in the absence of hairs. *P. submarinum* Wille is marine, but in 1926 Vischer described a second species (*P. basiliense*) from fresh water; the



var. *Brandii* Vischer (1933) is distinguished by the readiness with which filaments are formed on agar.

In liquid cultures of soil 7 there occurred single round or oblong cells (up to  $10\ \mu$  in diameter; Fig. 5, A, B) with a thin or thick membrane, as well as palmelloid stages of a dirty yellowish-green colour composed of similar cells embedded in structureless mucilage; all had a single parietal chloroplast, often lobed or reticulate and containing a pyrenoid. On transference to agar there was considerable multiplication with the production of similar palmelloid stages, as well as of pleurococcoid packets, and after a month some of the latter gave rise to branched filamentous growths (Fig. 5, E, F) surrounded by mucilage and consisting of up to eight cells ( $10\text{--}12\ \mu$  broad,  $\frac{1}{2}\text{--}1\frac{1}{2}$  times as long); the terminal cell was elongate (2–3 times as long as broad) and often showed division of pyrenoids so that growth is probably apical. Similar filamentous growths were also observed in liquid cultures (Fig. 5, C, D).

Zoospores were formed only in liquid cultures and in distilled water, and mostly arose from the filamentous stages, from 16 to 32 being produced in each cell (Fig. 5, c). They are liberated through an opening in the wall, are rounded or ovoid (Fig. 5, G),  $8\ \mu$  broad and  $8\text{--}10\ \mu$  long, and have a lobed chloroplast with a single pyrenoid; the eye-spot is situated near the middle of the cell and the four flagella are longer than the body. The single cells usually gave rise to 2–4 aplanospores which often remained in contact (Fig. 5, H).

The presence of an eye-spot and the readiness with which filaments are formed on agar confirms the reference to var. *Brandii*, although both the cells and the zoospores are broader than in Vischer's form.

## CONJUGALES

The principal Desmids found are.

*Cylindrocystis Brebissonii* Menegh. in soils 2, 3, 6–8, 14, 19, 20, 23, 24, an almost pure growth was found on a cutting near the station at Oxshott. The individuals from chalk soils are for the most part shorter than broad.

*Closterium pusillum* Hantzsch var. *monolithum* Wittr. was rather common in moist cultures of soils 6 and 19. This is the only species of the genus so far recorded from the soil (cf. James, 1935, p. 536). Chains of cells held together by mucilage were common in the moist cultures.

*Cosmarium cucurbita* Bréb., a common soil-form characteristic of non-calcareous sandy soils (16–19, 22), grows better in the moist cultures; conjugation frequent.

Apart from these, Desmids are sporadic. Moist cultures of soil 6 afforded *Euastrum sublobatum* Bréb. var. *subdissimile* West, *Cosmarium subcucumis* Schmidle, *C. decedens* (Reinsch) Racib., *C. anceps* Lund., *C. subcrenatum* Hantzsch, and *C. Holmiense* Lund. var. *integrum* Lund. The last was also found in soil 12, together with *C. etchachanense* Roy & Biss. As already



mentioned, *Zygogonium ericetorum* Kütz. was frequent in moist cultures of soil 21, as well as on the actual soil.

## II. XANTHOPHYCEAE (HETEROKONTAE)

*Pleurochloris terrestris* n. sp. (Fig. 6, A-E). Cells yellowish-green, spherical, oblong or three-sided, up to  $16\ \mu$  in diameter, with a thin membrane and a single parietal chloroplast, which is cup-shaped with rather uneven edges and contains a small round pyrenoid (Fig. 6, A); it assumes a blue colour with hydrochloric acid. The nucleus is in the middle of the cell. In reproduction the cells form 8 or 16 zoospores which are spherical,  $3.6-5\ \mu$  broad and provided with an eye-spot (Fig. 6, C); one flagellum is longer than the body, while the other is less than half its length. Aplanospore-formation is frequent. When the cells are transferred from agar to distilled water, gametes are formed, usually 8 per cell. They are globular or oblong, with a linear anterior stigma (Fig. 6, D), and are of slightly unequal size, the smaller about  $6\ \mu$  and the larger about  $9\ \mu$  in diameter; the cell-contents are obscured by oil-globules. The flagella are only slightly unequal. The zygotes are at first spherical, but later become quadrangular (Fig. 6, E).

This species, found in soil 6, is near *P. lobata* Pascher (1937-8, p. 347) in view of its single chloroplast with a pyrenoid, but it differs in the production of more numerous zoospores, in the shape of the latter, and the relative length of their flagella, and in the formation of aplanospores.

*P. acidophila* n. sp. (Fig. 6, G-I). Cells spherical,  $14-18\ \mu$  (occasionally up to  $24\ \mu$ ) in diameter, with a rather thick stratified membrane. Chloroplast single, with three deep lobes, one of which contains a small oval pyrenoid (Fig. 6, G). Older cells usually with a large central oil-globule. Swarmers are formed in large numbers by successive division (Fig. 6, H). They are spherical or oblong (Fig. 6, I),  $4\ \mu$  broad and  $4-6\ \mu$  long, with the chloroplast occupying about half the cell and almost equal flagella, both longer than the body; stigma posterior. Aplanospores formed in large numbers.

This species was found only in acid soils (21, 22) and differs from all others, except *P. anomala* James (1935, p. 539), in the relative lengths of the flagella, while it is distinguished from the latter species by the single-lobed chloroplast, by the presence of a pyrenoid, and by the shape of the zoospore.

*Vischeria stellata* (Chod.) Pascher, 1937-8, p. 559 (Fig. 6, M-O). Cells spherical or polygonal, single or in aggregates,  $8-14\ \mu$  in diameter, sometimes with angular projections on the wall, usually containing much reserve-food obscuring the chloroplast which appears to be lobed and without a pyrenoid. Multiplication by formation of 2-4 aplanospores (Fig. 6, O) in a cell; zoospores not observed. Very common in moist cultures of soils 16 and 18.

*Polyedriella helvetica* Vischer and Pascher; Pascher, 1937-8, p. 570 (Fig. 6, P-R). Cells single or in aggregates, spherical or polygonal, with thickened angles, usually  $8-10\ \mu$ , sometimes up to  $16\ \mu$  in diameter, with a large central crystal (Fig. 6, P, P<sup>1</sup>), older cells with orange-coloured oil; chloroplast single,

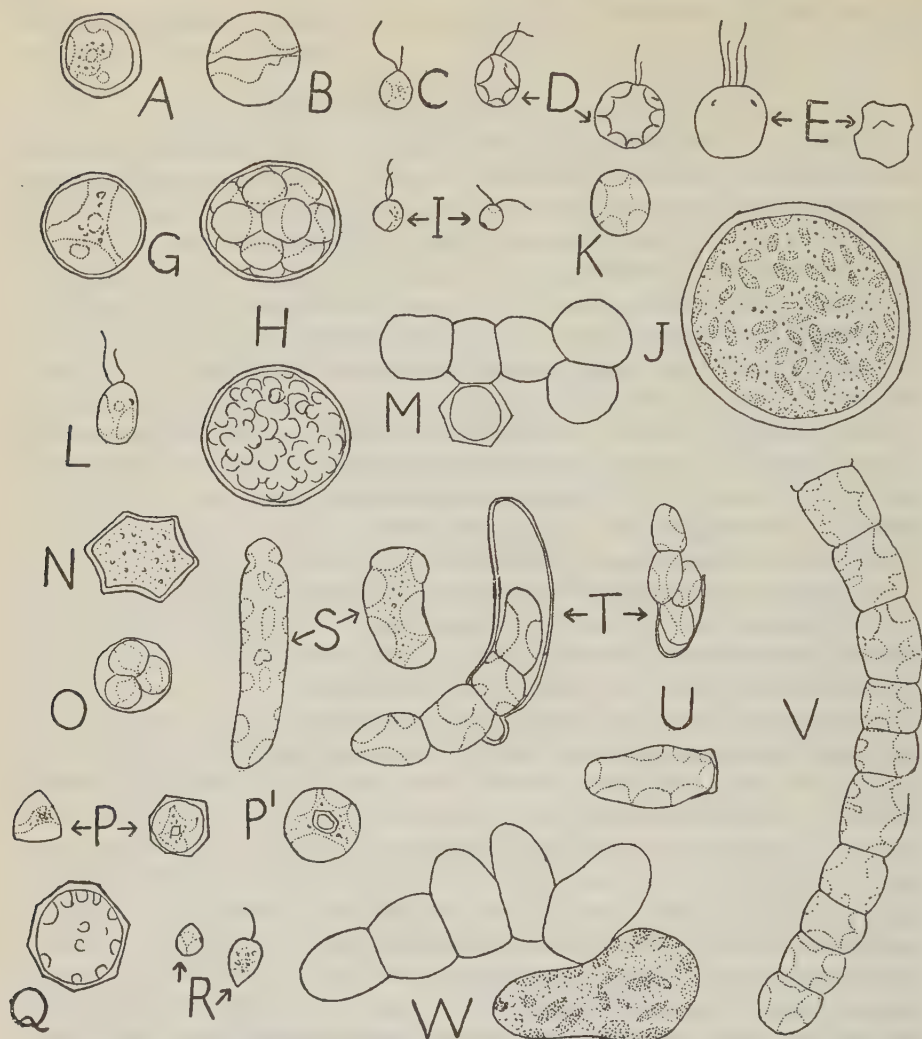


FIG. 6. A-E, *Pleurochloris terrestris* n. sp.; A, cell stained with aceto-carmine; B, dividing cell; C, zoospore; D, gametes; E, zygotes. G-I, *P. acidophila* n. sp.; G, single cell; H, stages in zoospore-formation; I, zoospores. J-L, *Botrydiopsis anglica* nov. nom., formae; J, mature, and K, young cells; L, zoospore. M-O, *Vischeria stellata* Chod.; M, N, cell-aggregate and single cell; O, division-stage. P-R, *Polyedriella helvetica* Vischer & Pascher; P, P', Q, cells of diverse types; R, zoospores, the left-hand one before liberation. S-U, *Bumilleriopsis Peterseniana* Vischer; S, two individuals; T, aplanospore-formation; U, young cell with part of parent-membrane attached. V, *Heterothrix quadrata* Pascher. W, *Heterococcus Chodati* Vischer. ( $\times 850$ .)

deeply lobed. Zoospores (Fig. 6, R), 1-2 per cell, spherical or ovoid, with a single flagellum longer than the body and a linear stigma near the anterior end. Autospores (2 per cell) frequent. Soils 4 and 11. This and the last are new records for Britain.

*Botrydiopsis anglica* nov. nom. (*B. minor* James, 1935, p. 537) *forma* (Fig. 6, J-L). The globular cells have a thick stratified membrane and the older ones possess many discoid or spindle-shaped chloroplasts (Fig. 6, J), which are yellowish-green and turn blue with hydrochloric acid; cells usually about  $19\ \mu$  broad, but may reach  $40\ \mu$ . Zoospores (Fig. 6, L), produced in large numbers, are set free by local gelatinization of the wall; they are highly metabolic,  $4-6\ \mu$  broad and  $8\ \mu$  long and contain two chloroplasts and an eye-spot situated near the anterior end; one flagellum is as long as the body, while the other is about  $2/3$ rd as long. Aplanospores are also formed, especially on agar.

This widely distributed form (soils 2-5, 7, 14, 15, 17, 19, 20, 23, 24) differs from the *B. minor* of James in the thick stratified membrane which is without local thickenings, in the less diffuse chloroplast, and in the amoeboid zoospores with an eye-spot. It is distinguished from all other species of the genus by the relative lengths of the flagella. Since the *B. minor* of Chodat has been shown to be a green alga (cf. Pascher, 1937-8, p. 394), it is necessary to give a new name to this frequent soil-alga.

*Bumilleriopsis Peterseniana* Vischer and Pascher; Vischer, 1936, p. 374; Pascher, 1937-8, p. 835 (syn. *B. brevis* Petersen, 1928a, p. 421) (Fig. 6, s-u). *Bumilleriopsis* is a genus based by Printz (1914, p. 50) on Gerneck's (1907, p. 241) *Ophiocytium breve*. The *B. brevis* described by Petersen (1928a, p. 421) from Icelandic and Danish soils differs in the fact that both ends of the cell are rounded and that there is a constriction near one end demarcating part of the wall as a lid; this was established as a separate species (*B. Peterseniana*) by Vischer (1936, p. 373).

The British material consisted of single cells (Fig. 6, s) or 3-celled filaments ( $6-10\ \mu$  broad and up to  $50\ \mu$  long) which are straight or curved and have a thin membrane. A constriction is present near one (Fig. 6, s) or both ends and here the wall is very thin. There are many parietal disc-shaped chloroplasts, while older cells contain white globules which do not give a fat-reaction. Reproduction is solely by means of aplanospores, 2-16 of which are formed in a cell; these are liberated by breaking of the membrane at the point of constriction, the part beyond often remaining attached at one side and appearing as a lid. The aplanospores (Fig. 6, t) are thin-walled and often develop the mature shape inside the parent-cell. Sometimes part of the wall of the latter remains around one end of an individual and gives rise to the thickening described by Petersen (Fig. 6, u). Soils 3, 5, 7, 9, 14, 20. A new record for Britain.

*Heterothrix exilis* (Klebs) Pascher. This is the commonest member of the class found in the soil (1, 2, 5, 7-14, 19, 20). The threads are on the whole rather narrow and the cells tend not to be so long as in the forms previously described; the chloroplasts vary in number from 2 to 4 per cell.

*Heterothrix quadrata* Pascher, 1937-8, p. 927 (Fig. 6, v). Slightly smaller than the form described by Pascher, with filaments  $8-9\ \mu$  broad, constricted between the cells and not easily fragmenting; cells shorter than or as long as



broad, with a considerable number of lenticular, closely packed chloroplasts; wall thin, without H-pieces; occasional longitudinal division. Soils 2, 4, 5, 14.

*Heterococcus Chodati* Vischer, 1936, p. 391 (*H. viridis* Chodat non Gerneck) (Figs. 6, w; 7, A). Isolated rounded cells or few-celled filaments (Fig. 6, w),

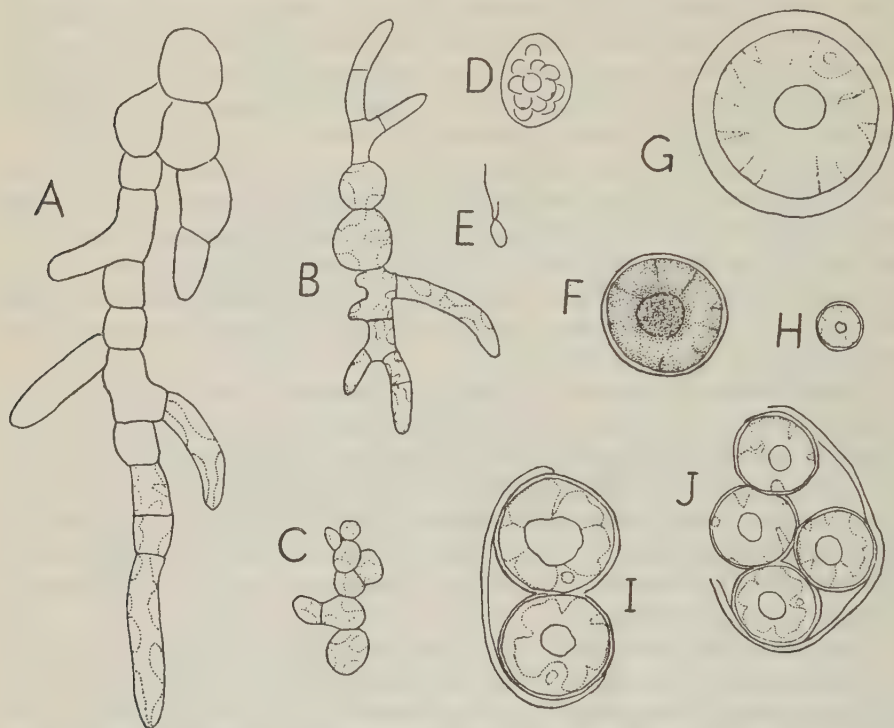


FIG. 7. A, *Heterococcus Chodati* Vischer. B-E, *H. caespitosus* Vischer; B, C filaments; D, zoospore-formation; E, zoospore. F-J, *Porphyridium aerugineum* Geitler; F, G, mature, and H, young cells; I, J, autospore-development. ( $\times 700$ .)

but no well-developed rosettes were found in liquid cultures. On agar more or less profusely branched irregular growths were produced (Fig. 7, A). Cells 6–14  $\mu$  broad, 10–28  $\mu$  long, thin-walled, with up to 10 disc-shaped chloroplasts. Swimmers, usually 8 per cell, rounded at the posterior and pointed at the anterior end, 3–4.5  $\mu$  broad, 7–8  $\mu$  long, slightly amoeboid, with a stigma at about the middle. Only the long flagellum was recognizable. Characteristic of chalk-soils (3, 5, 7, 14, 16 (?)).

*H. caespitosus* Vischer, 1936, p. 381 (Fig. 7, B-E). Usually found as profusely branched rosettes on the surface of the liquid cultures and as rounded cells or branched filaments (Fig. 7, B, C) on the bottom and sides, light yellow-green in colour; rounded cells in the centre of the rosettes up to 14  $\mu$  broad, cells of filaments 2.5–4  $\mu$  broad and 6–10  $\mu$  long. Any cell can produce up to 16 zoospores (Fig. 7, D), which are ovoid, 4–5  $\mu$  long, 2.5–3.5  $\mu$  broad, with an



eye-spot near the posterior end; one flagellum is twice as long as the body, while the other is about  $1/4$  as long (Fig. 7, E). Soils 5, 14, 20, 23.

### III. CHRYSOPHYCEAE

No detailed study of members of this class could be undertaken, but a few notes are appended on certain forms. In cultures of soils 12 and 19 there were present spherical or oblong cells (Chrysophyceae, 1; Fig. 8, J-L), usually embedded in groups in thin diffuent mucilage, up to  $8\ \mu$  broad and  $8-12\ \mu$  long, with a single parietal chromatophore with two or more very deep lobes and two contractile vacuoles (*c.v.*). Actual formation of swimmers was not observed, but slightly amoeboid motile cells rounded at both ends,  $4.5\ \mu$  broad and  $8\ \mu$  long, with a light brown chromatophore covering the posterior end probably belong here (Fig. 8, M). The two unequal flagella arise laterally a little below the anterior end, while the eye-spot lies just below their point of attachment.

Other palmelloid expanses (Fig. 8, E, F), found in soil 22 (Chrysophyceae, II), had spherical or oblong cells,  $8-12\ \mu$  broad and  $12-14\ \mu$  long, with a firm wall and two parietal chromatophores. The frequent swimmers (Fig. 8, G) were variable in shape,  $4-6\ \mu$  broad and  $10-12\ \mu$  long, with two golden-brown chromatophores, sometimes occupying only a very small portion of the cell, and two posterior contractile vacuoles; the two flagella were very unequal and the stigma minute. The cysts (Fig. 8, I) are spherical and colourless, with a thick wall ornamented with wart-like projections, sometimes surrounded by a wide sheath of diffuent mucilage (Fig. 8, H).

Large palmelloid growths containing numerous cells with a diameter of about  $4\ \mu$  were also found in soil 3 (Chrysophyceae, III).

### IV. BACILLARIOPHYCEAE

The most frequent of the Diatoms are: *Pinnularia borealis* Ehrb., one of the few found in acid soils (1, 2, 7, 10, 18, 21, 22); *Navicula atomus* (Naeg.) Grun. characteristic of chalk soils (2, 3, 5, 7, 14, 19, 20, 23), a larger form (up to  $14\ \mu$  long) than those previously described (Petersen, 1928a, p. 387); *Hantzschia amphioxys* (Ehrb.) Grun., the commonest of all (1-10, 12, 13, 15, 19, 21, 22); *Nitzschia palea* (Kütz.) W. Smith (3, 6, 7, 10, 12, 13, 15); and *Eunotia tenella* Kütz. (?) (common only in soil 16, also in 6 and 18). Species of *Achnanthes* are represented by *A. linearis* W. Smith (15) and *A. coarctata* Bréb. var. *elliptica* Krasske (1, 9), both often forming chains. Other Pinnularias are *P. interrupta* W. Smith f. *minutissima* Hustedt (Petersen, 1928a, p. 405; soils 4, 15); *P. parva* (Greg.) Cl. var. *Lagerstedtii* Cl. f. *interrupta* Petersen (1928a, p. 407), some individuals having the striae interrupted only on one side or not at all (soils 5, 7, 19?); *P. hemiptera* (Kütz.) Cl. (12); *P. viridis* (Nitzsch) Ehrb. (6, 8, 12, 15), the two last among the few larger diatoms found; and

*P. intermedia* Lagerst. (?), Petersen, 1915, p. 293; 1928a, p. 403 (15). Navicula is also represented by: *N. mutica* Kütz. var. *nivalis* (Ehrb.) Hust. (2, 3, 7); *N. cryptocephala* Kütz. var. *veneta* (Kütz.) Grun. (Petersen, 1915, p. 291) (7); *N. dicephala* (Ehrb.) W. Smith, small individuals, 13–20  $\mu$  long, 5–7  $\mu$

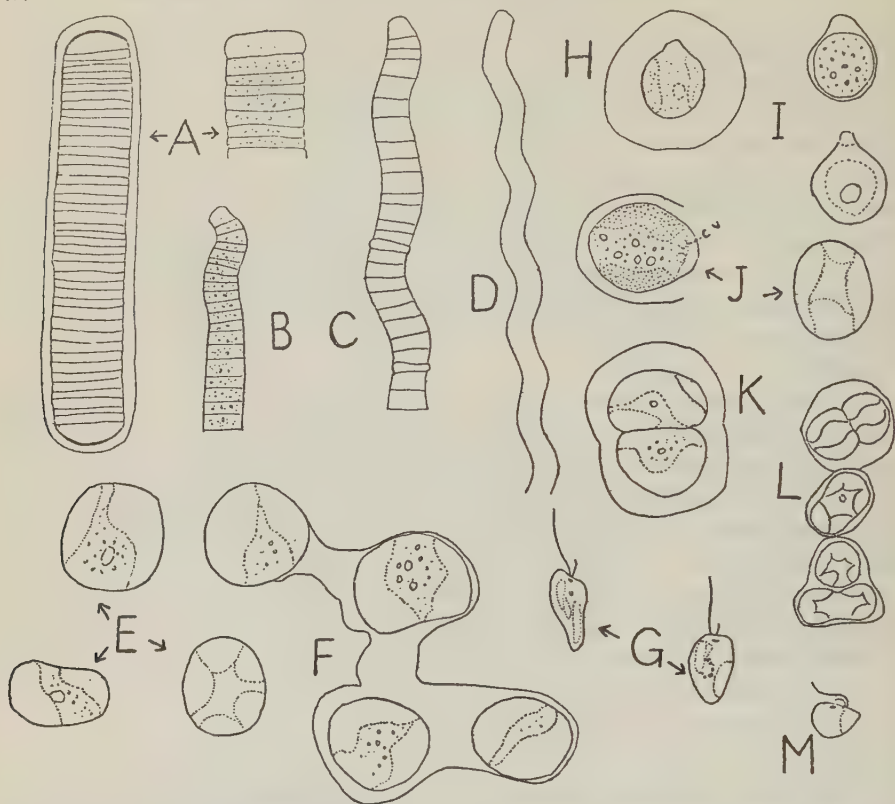


FIG. 8. A, *Crinalium magnum* n. sp. B–D, *Phormidium Hieronymusii* Lemm. E–M, Unidentified Chrysophyceae. E–I, Chrysophyceae II, from soil 22; E, single cells; F, aggregate stained with methylene blue; G, swarmer; H, I, cysts. J–M, Chrysophyceae I, from soils 12 and 19; J, single cells; K, L, groups; M, swarmer. c.v., contractile vacuole. (A–C  $\times 600$ ; D  $\times 450$ ; G, M  $\times 900$ ; the rest  $\times 1,500$ .)

broad (3, 7); and *N. cincta* (Ehrb.) Kütz. (12). *Diploneis elliptica* (Kütz.) Cl. was found only in soil 12, which also furnished the only *Amphora* (*A. Normani* Rabenh.) and two *Nitzschias* (*N. Tryblionella* Hantzsch var. *debilis* (Arnott) A. Mayer, Petersen, 1928a, p. 416; *N. amphibia* Grun.), the first found also in soil 4. *N. vermicularis* (Kütz.) Grun. var. *terrestris* Petersen (1928a, p. 418), which is distinguished from the type by its smaller size (50–55  $\mu$  long, 4.5–5  $\mu$  broad), the slight concavity of the median part, and the frequent elongation of the carinal dots in the direction of the long axis, occurred in soil 6. Somewhat doubtful individuals of *Surirella linearis* W. Sm. were found in soils 6 and 15.

## V. MYXOPHYCEAE (CYANOPHYCEAE)

The only member of Chroococcales met with was *Chroococcus minutus* (Kütz.) Naeg., found mostly in moist cultures and only in calcareous soils (1, 4, 6, 9). Oscillatoriaceae are represented chiefly by species of Phormidium, viz. *P. foveolarum* f. *major* Elenkin (Geitler, 1932, p. 999), very common in most chalk soils (2-4, 6, 7, 9, 10, 13) and usually with *Chlorochytrium paradoxum* embedded in the stratum; *P. autumnale* (Ag.) Gom. (2-7, 9, 12, 13, 15, 19, 20); *P. uncinatum* Gom. (13); *P. tenue* (Menegh.) Gom. (9, 12, 13); and *P. Hieronymusii* Lemm. (Geitler, 1932, p. 1017). The last is a new record for Britain. It has bright blue-green filaments, up to 9  $\mu$  broad (cells 1.5-2.5  $\mu$  long), more or less regularly undulate throughout or only near the apex (Fig. 8, c, d) and with a thin colourless sheath. The end of the trichomes is more or less markedly attenuated, with an end-cell up to 3.5  $\mu$  long, without a calyptra. It was found in soils 2, 3, and 7.

*Microcoleus vaginatus* (Vauch.) Gom. was present only in soils with a high percentage of  $\text{CaCO}_3$  (3, 5, 7). In soil 3 the alga showed the annular constrictions described for *M. annulatus* (Fritsch and Rich, 1924, p. 359), but on moistening the culture with distilled water these disappeared showing that they were due to desiccation. Soil 13 contained a number of other Oscillatoriaceae, viz. *Oscillatoria formosa* Bory; *Microcoleus paludosus* (Kütz.) Gom.; and *Schizothrix arenaria* (Berk.) Gom. Of interest is the discovery of a new species of Crinalium (Crow, 1927) which is characterized by its strap-shaped filaments. In *C. endophyticum* Crow, an endophyte within the mucilage of *Aphanocapsa*, the filaments are curved like a hair-pin and for the most part spirally twisted; they have a thin sheath. The genus is closely allied to Gomontiella, but in Crinalium the filaments are not inrolled at their margins. The new species (*C. magnum*), which has straight flattened trichomes (Fig. 8, A), never spirally twisted or bent, occurred in soils 1 and 9, the isolated threads being usually buried in the sand of the moist culture. The trichomes possess a narrow sheath of diffuent mucilage, which is closed at the ends and visible only after staining; they reach a length of 0.5 mm., although most of them are shorter, and the trichomes are slightly constricted at the non-granulate septa. The ends are not attenuated, the end-cell being slightly longer than the others, with a convex and slightly thickened outer margin. The cells are up to 18  $\mu$  broad and 1.5-3  $\mu$  long.

The commonest species of Nostoc was one doubtfully identified as *N. commune* Vauch.; this occurred in soils 2, 3, 5, 7, 9, 10, 13. Apart from this, *N. paludosum* Kütz. (?) was found in soils 4, 7, 10, and *N. humifusum* Carm. in soil 11. The only species of Anabaena found were *A. variabilis* Kütz. (soils 1, 9), with akinetes 14-17  $\mu$  long, and *A. torulosa* (Carm.) Lagerh. (soil 15). *Cylindrospermum* was represented by *C. stagnale* (Kütz.) Born. & Flah. (soil 15) and *C. alatosporum* Fritsch (1917, p. 578) (soil 6), both with akinetes; those of the former were sometimes barrel-shaped, 10-11  $\mu$  broad and up to



30  $\mu$  long, while in the latter the radially striated outer membrane of the spore was continued over the end adjacent to the heterocyst. A very common blue-green form, found mostly in calcareous soils (1, 2, 4-6, 9, 10, 13, 15), is *Tolypothrix tenuis* Kütz. f. *terrestris* Petersen (1923, p. 306); the sheath was not as thick as in Petersen's figures. *Plectonema nostocorum* Born. occurred in soils 5, 14, and 20.

## VI. RHODOPHYCEAE

*Porphyridium aerugineum* Geitler (1924, p. 362) appears as single spherical cells (fig. 7, F, G), reaching a diameter of 60  $\mu$ , with a wall up to 4  $\mu$  in thickness. The axile chromatophore has 2-4 shallow lobes in the young (Fig. 7, H) and many deep ones in the older cells (Fig. 7, F, G); the colour is blue-green in the liquid cultures, but grass-green on agar. The large pyrenoid has a prominent starch-sheath (Fig. 7, F), while the nucleus (Fig. 7, G) lies in a cleft between the lobes of the chromatophore. 2, 4, or 8 autospores (Fig. 7, I, J) are formed in a cell and set free by breaking of the wall on one side. The best growth was found in soil 20, but the species was also present in soils 5 and 14. It has previously been recorded from soil and water.

*Euglena mutabilis* Schmitz, the only representative of its class, was found in soils 7, 8, 17, 21-4; it grows best in acid soils.

## SUMMARY

This communication deals with the algae present in the soils considered in Part I. The following genera are newly recorded for the British Isles: *Macrochloris*, *Borodinella*, *Muriella*, *Fernandinella*, *Interfilum*, and *Pseudendoclonium* among Chlorophyceae; *Vischeria*, *Polyedriella*, and *Bumilleriopsis* among Xanthophyceae. Reasons are given for merging *Hypnomonas* in *Chlorococcum*, and the occurrence of *Trochiscia*-like stages in the life-cycle of the latter are described. The new species described include *Muriella magna*, *Dictyosphaerium terrestre*, *Pleurastrum terrestre*, *Pleurochloris terrestris*, *P. acidophila*, *Crinalium magnum*, five species of *Chlamydomonas*, and two of *Carteria*.

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# Studies in the Comparative Morphology of the Algae<sup>1</sup>

## I. Heterotrichy and Juvenile Stages

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With four Figures in the Text

IN the numerous instances of parallel development among Algae reported during the present century (cf. Fritsch, 1935, p. 26), stress has, in the main, been laid on the simpler types of plant-body, and the rather considerable degree of correspondence shown by the more advanced forms has been largely ignored. This parallelism is, however, appreciable, and it justifies the conclusion that the later stages of the evolution of the algal thallus have, like the earlier ones, followed essentially the same trend in those classes in which more elaborate multicellular types occur. The most significant instances of morphological parallelism are those which are provided by forms exhibiting what I have termed the heterotrichous habit (Fritsch, 1939). This reappears, in diverse modifications of much the same type, in a considerable number of algal classes and is evidently an evolutionary stage which was reached wherever a multicellular differentiation was attained. So far as present knowledge goes, it is not well represented in the Xanthophyceae (Heterokontae) and Chrysophyceae.

In its most generalized form this habit is exemplified by the heterotrichous filament as illustrated by *Stigeoclonium* and *Trentepohlia* among Chlorophyceae, *Ectocarpus* (Fig. 1, A) among Phaeophyceae, *Erythrotrichia* (Fig. 1, T) among Bangiales, and *Acrochaetium* (*Chantransia*) (Fig. 1, F) and *Rhodochorton* among Florideae. It is also widely represented among Cyanophyceae in the Stigonematales, while in the Pleurocapsales, the Chrysophyceae, and Xanthophyceae only what appear to be derived types are so far known. The essential characteristic of the heterotrichous filament is the successive development of the plant-body in two perpendicular planes (Fig. 1, A, F, H, T). As a general rule it is the creeping or prostrate system (*b*) that develops first (Cienkowski, 1876, p. 18; Huber, 1892 *a*, p. 322), extending in the horizontal plane and serving as the primary means of attachment to the substratum. It is, however, composed of cells containing chromatophores and

<sup>1</sup> The green types referred to in the following are fully illustrated in Fritsch, 1935, pp. 248 et seq.

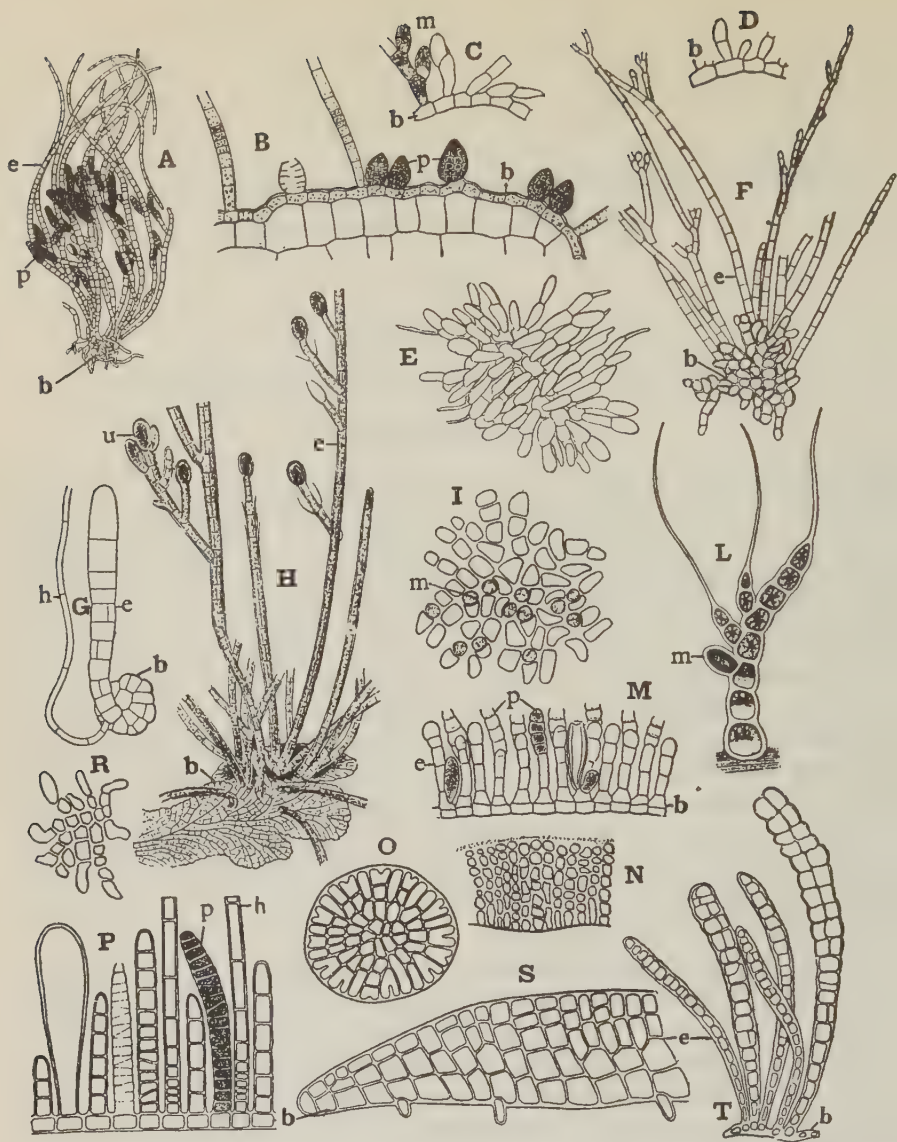


FIG. 1. Heterotrichous types. A, *Ectocarpus cylindricus* Saunders, forma, group of heterotrichous filaments. B, *E. Battersii* Born., reduced epiphytic type. C-E, *Acrochaetium humile* (Rosenv.), reduced epiphytic type; C, D, in vertical section; E, adult plant from above. F, *A. attenuatum* (Rosenv.), heterotrichous filament, prostrate system seen from below. G, *Cladostephus verticillatus* Lyngb., young plant with prostrate system, hair (h) and first erect shoot (e). H, *Sphacelaria olivacea* C. Ag., part of mature plant. I, *Erythrocladia irregularis* Rosenv., discoid type. L, *Acrochaetium crassipes* Boerges. var. *longiseta* Boerges., erect type. M, *Myrionema strangulans* Grev., vertical section of part of plant. N, *Oncobyrsa rivularis* Kütz., vertical section of part of crust. O, P, *Ascocyclus orbicularis* Magnus; o, primary disc; p, mature plant in section. S, *Peyssonnelia Dubyi* Crouan, vertical section of edge of crust. T, *Erythrotrichia obscura* Berth., heterotrichous filament. b, prostrate, and e, erect systems; h, hair; m, monosporangium; p, pluri-, and u, unilocular sporangia. (A after Setchell & Gardner; B, L, T after Boergesen; C-F after Rosenvinge; G, M after Sauvageau; H after Reinke; I, O, P, S after Kylin; N after Geitler.)



constitutes the first photosynthetic system of the plant. From the creeping system there originates the erect one (*e*) by outgrowth of more or less numerous cells of the former in a plane perpendicular to its direction of expansion. The relative degree of development of the two systems varies appreciably in different, and probably even in the same, species of *Stigeoclonium*, and this is no doubt also true of *Ectocarpus* and *Acrochaetium*. In *Stigeoclonium tenue* and other species (Huber, 1892 *b*, p. 274; Berthold, 1878, p. 199) the prostrate system is but a loosely ramified thread, while in *S. farctum* (Berthold, 1878, p. 201; Fritsch, 1903, p. 376) it is a richly branched expanse or even a compact disc with numerous threads coalescent to form a one-layered stratum, at the periphery of which the free ends of the branches may or may not project (Fig. 74, c, d, in Fritsch, 1935, p. 250). In *S. tenue* the erect system is well developed and extensively branched in different planes, whereas in forms with an elaborate prostrate disc the former may be reduced to a few short branches or in places be represented only by hairs (*S. prostratum*, Fritsch, 1918, p. 531). Similarly in *Trentepohlia umbrina* the erect system is often very little developed, while the majority of the species of *Coleochaete* contrast with *C. pulvinata* in the complete elimination of all erect threads.

Little is known about the degree of development of the prostrate system in the ordinary species of *Ectocarpus*, but quite a number have been described (e.g. *E. Battersii* Gom., Fig. 1, B; *E. elachistaeformis* Heydr., Boergesen, 1914, p. 174) in which the erect system is scantily represented and the sporangia (*p*) arise directly on the prostrate base. Among the species of *Acrochaetium*, where more data as to the early stages of germination are available (Bornet, 1904, p. xviii; Rosenvinge, 1909, p. 81; Chemin, 1937, p. 302), the relative importance of the two systems evidently varies considerably and many species with an ill-differentiated erect system (Fig. 1, C-E), parallel to those found in *Ectocarpus*, are known. Moreover, in this genus there are both heterotrichous types (e.g. *A. Daviesii*, *A. attenuatum*) and others in which growth is entirely erect from the first (e.g. *A. crassipes*, Fig. 1, L); on the other hand, all species of *Rhodochorton* are definitely heterotrichous. Among *Bangiales*, *Erythrotrichia* shows a parallel condition to that found in *Acrochaetium*, *E. Boryana* and *E. obscura* (Fig. 1, T) possessing heterotrichous filaments, while in *E. carnea* the growth is solely erect. It seems that a similar contrast exists among the species of *Stigeoclonium* (cf. Gay, 1891, p. 42; Godward, 1942) and possibly also among those of *Ectocarpus*.

This raises the question as to the relative status of the heterotrichous and non-heterotrichous filaments, which respectively constitute the chief basis of distinction between *Chaetophorales* and *Ulotrichales* among *Chlorophyceae*. There is an evident resemblance between *Stigeoclonium* and *Ulothrix* in cell-structure and reproduction, despite the marked difference in habit. This may result from a derivation of *Chaetophorales* and *Ulotrichales* from a common ancestry, but it is perhaps equally possible that the latter are an offshoot from the former along a line in which the prostrate system has been

lost.<sup>1</sup> Such an evolutionary sequence is probable among Bangiales, where the Bangieae (*Bangia* and *Porphyra*) usually afford no evidence of heterotrichy, although it is recognizable in *P. naiadum* (Hus, 1902, p. 180; Knox, 1926). It is to be noted that, both in Ulotrichales and Bangiales, the absence of a prostrate system is associated with increased somatic development (Ulvaceae, *Porphyra*) and in the former with a progression from isogamy to oogamy (*Cylindrocapsa*).

Whatever be the relation of the non-heterotrichous Ulotrichales to the Chaetophorales, there is no doubt that in the latter order advance in vegetative differentiation is associated with disappearance of the prostrate system, as exemplified by *Draparnaldia* and *Draparnaldiopsis*; so far as present knowledge goes, the plants of the former are altogether erect in their growth (Berthold, 1878, p. 209). This elimination of the prostrate system appears to mark a definite trend in all heterotrichous series and is very evident, although on a wider scale, in Phaeophyceae and Florideae. In the former class heterotrichy is manifest in the early stages of development of the diploid sporophyte in practically all Ectocarpales that have been investigated (see Figs. 2, A-C and 3, A, D, E; cf. below); it is also widespread in many Sphacelariales (*Sphacella*, *Sphacelaria*, Fig. 1, H; *Cladostephus*, Fig. 1, G). On the other hand, in the Desmarestiales and Sporochnales and in the advanced parenchymatous Phaeophyceae (Laminariales, Fucales) there is no prostrate system, and growth of the sporophyte is erect from the first. Heterotrichy, however, persists in the gametophytic stages of most Brown Algae (Fig. 3, H, I, L), a fact which is of importance in elucidating the relative status of haploid and diploid phases in Ectocarpales (see article 2). Among Florideae, the majority of the haplobiontic Nemalionales agree with the Ectocarpales in possessing a primary heterotrichous stage (cf. e.g. Chemin, 1937, p. 310), and this is also true of some of the diplobiontic Cryptonemiales and Gigartinales (Figs. 2, L, N; 3, C). In many of the two last, as well as in Rhodymeniales, however, heterotrichy is only indicated, the spore at an early stage segmenting by horizontal as well as vertical walls (Fig. 2, F, G, M), while in the highly specialized Ceramiales (Fig. 2, H, I, O) all traces of it have been lost. It would, therefore, seem that in the further evolution of heterotrichous forms the more highly specialized types have eliminated the prostrate system, this occurring either only in the diploid phase when there is heteromorphic alternation (Sporochnales, Desmarestiales, Laminariales), or in both diploid and haploid phases when there is isomorphic alternation (Dictyotales, diplobiontic Florideae).

In nearly all heterotrichous series an opposite condition is manifest, viz. the reduction or suppression of the erect system, with the development of prostrate epiphytic or lithophytic types. This is probably often a culmination of the tendency seen among the heterotrichous filamentous forms towards a reduction of the erect system (cf. above). Such more or less completely

<sup>1</sup> Certain peculiarities in the germination of the zoospore of *Ulothrix* (Klebs, 1896, p. 306; Gross, 1931, p. 225) are perhaps significant in this connection.

prostrate types are illustrated by the considerable assemblage of genera grouped as Prostratae among Chaetophoraceae (Fritsch, 1935, p. 258), by genera like Streblonema, Ascocyclus (Fig. 1, o, p), and Mikrosyphar among Ectocarpaceae, and by Erythrocladia (Fig. 1, i) among Bangiales; among Florideae no certain parallel types are so far known. It is to be noted that the degree of reduction varies. Thus, among Prostratae some genera (Chaetonomia) produce a few erect branches, others bear only hairs on the prostrate system (Aphanochaete, Bolbocoleon), while still others lack all erect growth (Pringsheimia, Ulvella). Forms like Chaetonomia and Aphanochaete are almost certainly reduced (i.e. specialized), as indicated by the advance to anisogamy or oogamy, but it remains an open question whether all completely prostrate types are to be interpreted in this sense. With our present knowledge, the possibility cannot be ruled out that the first step in the evolution of the heterotrichous habit may have been a branched creeping filament or expanse attached throughout to the substratum, a type of plant-body admirably suited to effective photosynthesis. From such a growth erect threads may have arisen secondarily. In other words, it may be that genera like Protoderma and Ulvella, Thallochrysis (Chrysophyceae), Heteropedia (Xanthophyceae; Pascher, 1937, p. 66), and Erythrocladia (Bangiales) represent the most primitive state of the heterotrichous habit, a possibility which is not incompatible with the existence of prostrate types that have arisen by reduction from a heterotrichous filament.

A special modification of the heterotrichous habit is that in which most or all of the cells of the prostrate system bear erect, little-branched filaments, usually of no great height and commonly more or less completely coalescent so that crusts or cushions are produced. This condition is rare among Chaetophorales (Pseudopringsheimia), but is well seen in the Myrionemataceae (Myrionema, Fig. 1, m, Ralfsia, Lithoderma) among Phaeophyceae, in the Corallinaceae (Melobesia, Lithophyllum), Cruoriaceae, and Squamariaceae (Fig. 1, s) among Florideae, and in many Pleurocapsales (Oncobyrsa, Fig. 1, n, Radaisia) among Myxophyceae; it also occurs, here in a somewhat modified form, in Phaeodermatium (Pascher, 1925, p. 518) among Chrysophyceae. It is at present difficult to settle whether this habit represents a special development or whether, as has been held for the Myrionemataceae (Kuckuck, 1929, p. 8), it results from reduction from more elaborate types. The latter is likely for Myrionema, which appears to show heteromorphic alternation (Kylin, 1934, p. 7). Among diverse Florideae (Gloeosiphonia, Fig. 3, c; Dumontia; Platoma, Fig. 3, b) the prostrate system of the primary heterotrichous stage bears numerous short, often coalescent, erect filaments, affording a crust-like attachment organ which shows much similarity with the encrusting thalli of Cruoriaceae and Squamariaceae (cf. Fig. 1, s).

The fact that lends particular significance to the heterotrichous habit, and indicates that it has been an important stage in the evolution of the more elaborate types of thalli found only in Brown and Red Algae, is that all the



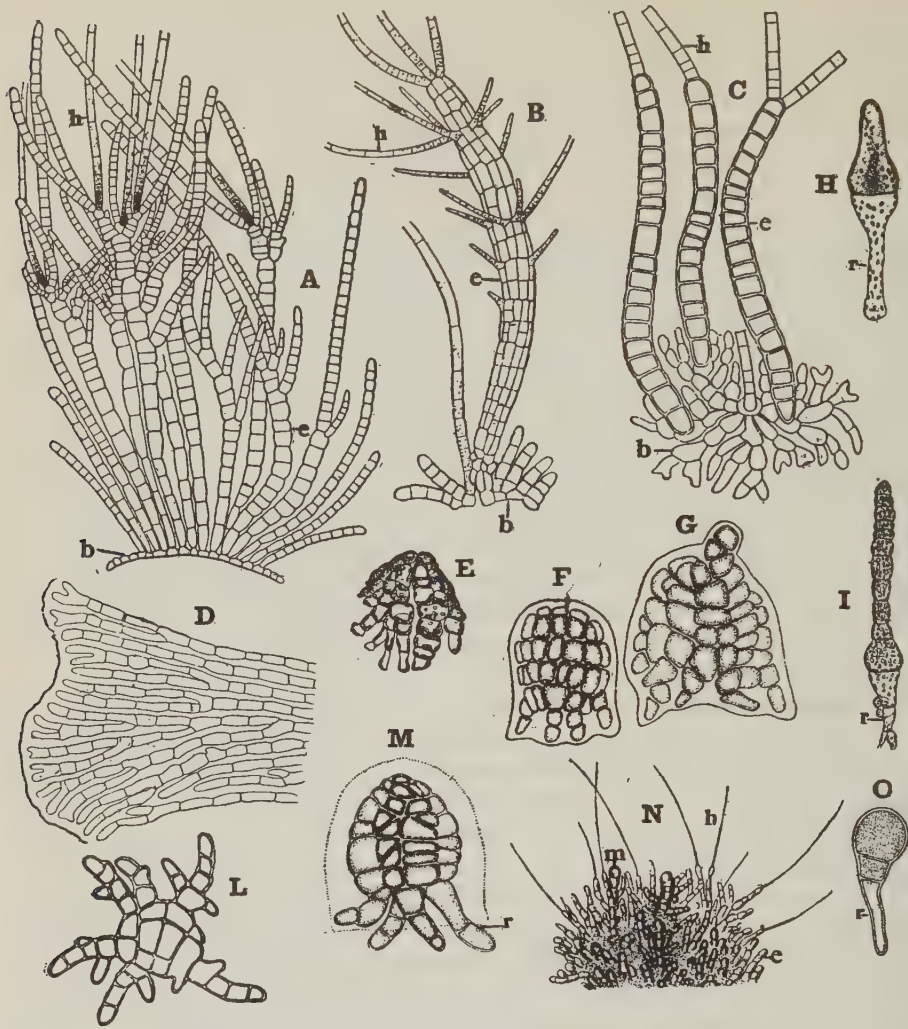


FIG. 2. Juvenile stages. A, *Castagnea fistulosa* Derb. & Sol. B, *Scytosiphon lomentarius* (Lyngb.) J. Ag. c, *Desmotrichum undulatum* Reinke. D, *Myrionema strangulans* Grev., part of basal system. E, *Lomentaria clavellosa* (Turn.) Gaill. F, G, *Cystoclonium purpureum* (Huds.) Batt. H, I, *Dasya arbuscula* Ag. L, *Bonnemaisonia asparagoides* (Woodw.) Ag., primary disc of germling. M, *Chylocardia kaliformis* (G. & W.) Hook. N, *Dudresnaya* sp., mature shoots (m) arising from prostrate system of young plant. O, *Ceramium* sp. b, prostrate, and e, erect systems; h, hair; r, rhizoid. (A, B after Kuckuck; D after Sauvageau; E, H, I, N after Killian; the rest after Kylin.)

evidence goes to show that it is from it as a starting-point that the latter have originated. Thus, the primary stage in the development of all the more advanced Ectocarpales is almost invariably of the nature of a heterotrichous filament. The prostrate base, commonly in the form of a more or less compact disc clearly composed of radiating filaments (cf. Fig. 2, C, D), appears always



to be the part formed first and acts as the primary photosynthetic system of the plant. It is from the erect threads arising from it that the mature thallus is progressively elaborated. A single thread may take the lead (*Mesogloea*, *Acrothrix*, *Spermatochnus*), or the mature thallus may result from the simultaneous upgrowth of a number of adjacent threads, giving the multiaxial construction so well exemplified by *Castagnea* (Fig. 2, A) and other *Mesogloeaceae*. In the forms just mentioned (the haplostichous *Ectocarpales* of Kuckuck, 1929, p. 6) the mature thallus consists of compacted branches of the single or several upgrowing threads, a construction analogous to that of most *Florideae*. In the polystichous *Ectocarpales* of Kuckuck, however, single erect filaments of the heterotrichous stage undergo septation of their cells in various planes to produce the mature thallus, giving a striking series of forms with a parenchymatous construction (*Scytosiphon*, Fig. 2, B; *Desmotrichum* Fig. 2, C; *Dictyosiphon*, &c.), a feature the evolutionary significance of which will be dealt with in the third article of this series. A similar derivation from a primary heterotrichous stage is manifest in *Tilopteridales* (Reinke, 1889, p. 108; Sauvageau, 1928*b*, p. 74) and many *Sphacelariales*. The early stages of *Cladostephus verticillatus* (Fig. 1, G), so clearly described by Sauvageau (1914, p. 566), indicate a similar origin for this relatively advanced type. There can, therefore, be no question that heterotrichy underlies the construction of the diverse orders of *Phaeophyceae* just mentioned, although, as stated above, no trace of it survives, except in the gametophytes, in the more specialized oogamous orders.

The same is true among *Florideae*, where, however, no parenchymatous types occur and the thallus is always built up of branching threads, giving the uni- or multi-axial construction met with throughout the class. The derivation from a primary heterotrichous stage is patent in numerous *Nemalionales* (*Batrachospermum*, *Lemanea*, *Nemalion*, *Atractophora*, Chemin, 1937, p. 321; cf. also Fig. 2, L), and Oltmanns (1922, p. 254) has emphasized the marked similarity between these stages in the uni-axial *Gloeosiphonia* (Fig. 3, c) and the multi-axial *Platoma* (Fig. 3, B) among *Cryptonemiales* and *Gigartinales* respectively. Killian's (1914), Kylin's (1917), and Chemin's (1937) studies of the germination of the spores in diverse *Florideae* indicate how widespread such primary heterotrichous stages are among the less specialized orders. There is this difference from the condition usual in *Ectocarpales* that the majority of the cells of the prostrate base commonly grow out into short and often coalescent erect threads, affording a crust which frequently becomes the perennial part of the plant (*Dumontia*). The same is true of some *Sphacelariales*.

A feature that is of considerable importance for the interpretation of the life-cycle of the *Ectocarpales* is the frequent presence of reproductive organs in the primary stages, which under these circumstances may show a tendency to persist for long periods in this condition. This phenomenon has been recorded in *Myriotrichia* (Kuckuck, 1899*d*, pp. 61, 71), *Punctaria* (Sauvageau,

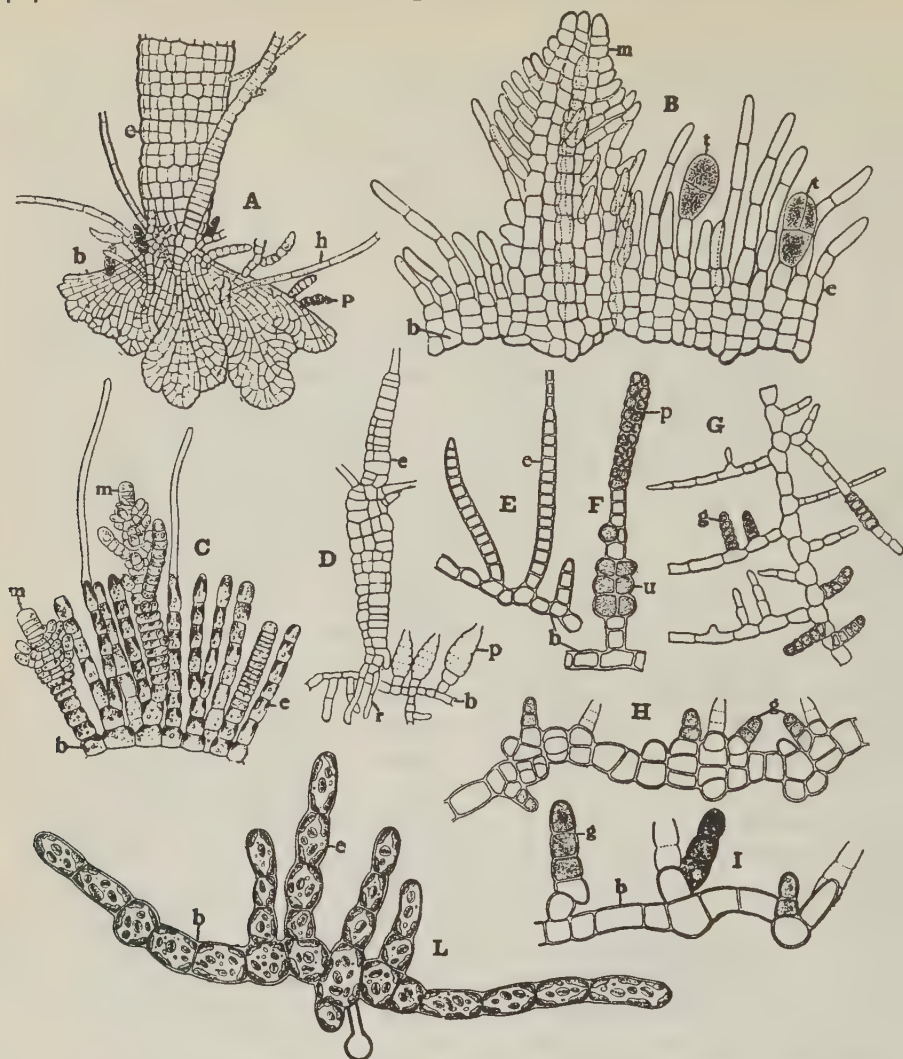


FIG. 3. A, *Asperococcus scaber* Kuck., young stage, plurilocular sporangia on basal system. B, *Platoma Bairdii* (Farl.) Kuck., vertical section of basal stratum, with beginning of mature frond (*m*) and tetrasporangia (*t*). C, *Gloeosiphonia capillaris* (Huds.) Carm., juvenile stage with two developing fronds (*m*). D, *Punctaria latifolia* Grev., juvenile stage, plurilocular sporangia on basal system. E-G, *Litosiphon pusillus* Harv.; E, plethysmothallus with erect threads developing into mature fronds; F, ditto, early fertility of erect thread; G, gametophyte. H, *Stilophora rhizodes* J. Ag., gametophyte. I, *Spermatocnus paradoxus* Kütz., gametophyte. L, *Alaria crassifolia* Kjellm., male gametophyte. *b*, prostrate, and *e*, erect systems; *g*, gametangia; *m*, mature frond; *p*, plurilocular sporangia. (A-C after Kuckuck; D after Sauvageau; E-H after Kylin; I after Papenfuss; L after Kanda.)

1929, p. 338; Fig. 3, D), and *Asperococcus* (Kuckuck, 1899c, p. 51; Fig. 3, A) among Ectocarpales, where in such instances uni- or plurilocular sporangia are found on the prostrate system. *Sphacelaria olivacea* is at times represented

solely by the basal crust which bears the unilocular sporangia directly, a stage described by Kuckuck (1894, p. 232) as a distinct genus (*Sphaceloderma*). Similarly in *Erythrotrichia discigera* the prostrate base is often alone represented (Schmitz's *Erythropeltis*, 1897, p. 313) and produces monosporangia. A comparable condition is known in diverse Florideae (e.g. *Platoma*, Fig. 3, B), where tetrasporangia are borne on the erect threads of the primary crust. The so-called 'Chantransia-stages' of *Batrachospermum* and *Lemanea* are nothing else than the primary heterotrichous stages, which in the former propagate abundantly by monospores. Under certain conditions (feeble illumination) these stages may persist for long periods without giving rise to the adult thallus. They are, however, no more 'protonemal' in nature than are the early heterotrichous stages of a *Nemalion* or *Dudresnaya*. The fact that the mature *Batrachospermum*- or *Lemanea*-thallus usually arises as a lateral branch of one of the erect threads is of no significance, the more as it occasionally grows out direct from the prostrate system (Brand, 1895, p. 283), and that in *Dudresnaya* (Killian, 1914, p. 240) the former condition obtains.

It is to Sauvageau (1928 a, 1932) that we are indebted for the demonstration that the more advanced Ectocarpales, with prevalently annual sporophytic thalli, persist during the period of absence of the macroscopic plant as minute filamentous stages which are heterotrichous in nature. These he has described from cultures in a considerable number of the members of this order, and the data show that they are invariably ectocarpoid or myrionemoid in form (Sauvageau, 1929, 1931; cf. also Kylin, 1933, 1934). It is possible that in nature they may attain to somewhat larger dimensions, and some of them at present perhaps figure as species of *Ectocarpus*, *Myrionema*, or allied forms. The swarmers from the two types of sporangia (uni- and plurilocular), borne on the macroscopic thalli of most Ectocarpales, give rise to practically similar growths, and these usually bear plurilocular sporangia (Fig. 3, G-I). The swarmers produced in the latter reproduce these stages and, in diverse species, a number of successive generations have been reared in cultures. On the other hand, in certain instances these swarmers have proved to be gametes and, when this is so, the filamentous stages in question are *always* derived from the swarmers of the unilocular sporangia of the macroscopic plant; these stages are therefore gametophytes and the evidence indicates that, even when no sexual fusion has been observed, they are potential gametophytes (see article 2 of this series). The sporangia on the minute filamentous stages grown from the swarmers of the plurilocular sporangia of the macroscopic plant, on the contrary, *invariably* produce *asexual* swarmers; these stages are, moreover, peculiar in the fact that they occasionally bear unilocular sporangia as well, although such sporangia never occur on the actual or supposed gametophytes.

There is every reason to believe that, apart from certain exceptions, the swarmers from the unilocular sporangia of the macroscopic plant give rise to haploid, those from the plurilocular sporangia to diploid, filamentous stages.



The latter may well be designated by the term *plethysmothallus* introduced by Sauvageau, although he applied the term indiscriminately, and, as the writer believes, incorrectly (Fritsch, 1939, p. 128), to all filamentous growths, whether derived from uni- or plurilocular sporangia of the sporophyte, so long as the swarmers produced in their sporangia afforded no evidence of sexuality. Two kinds of data speak for the interpretation of the filamentous stages, produced from the swarmers of the plurilocular sporangia of the macroscopic thallus, as diploid. In the first place, we have the occasional presence of unilocular sporangia, already referred to, on the plethysmothalli, and there is no evidence as yet forthcoming that such sporangia are ever produced on anything but a diploid stage. In the second place, the erect filaments of these plethysmothalli have in several instances been found sooner or later to give rise to mature sporophytes (Sauvageau's protonemata) and, when this is so, they may be altogether sterile (Fig. 3, E) or bear plurilocular (and unilocular) sporangia as well (Fig. 3, A, D, F).

I am of the opinion that all the evidence indicates that not more than two types of minute filamentous growths are to be distinguished among the Ectocarpales, viz. gametophytes and plethysmothalli, the former possibly often propagating apogamously for long periods, the latter propagating by the swarmers produced in their plurilocular sporangia, but sooner or later initiating a new sporophyte. The plethysmothalli are in fact nothing else than the juvenile heterotrichous stages of the sporophyte, directly comparable with the Sphaceloderma-stage of *Sphacelaria olivacea* and analogous to the 'Chantransia-stages' of the haploid *Batrachospermum*. Like the last, they may apparently, under given conditions, persist for long periods in the arrested condition until circumstances become favourable for the development of the adult thallus. The marked degree of correspondence between gametophytes and plethysmothalli is the outcome of the fundamental heterotrichy and isomorphic derivation (see article II) of both generations.

A somewhat anomalous position among the Brown Algae showing heteromorphic alternation is occupied by Cutleria, not only because of its evident close affinity with the isomorphic Zanardinia, but also because, unlike other heteromorphic Phaeophyceae, both generations here are macroscopic, although markedly different in appearance and structure. The haploid Cutleria-stage is devoid of all prostrate system and possesses a thallus formed by the coalescence of more or less numerous filaments with trichothallic meristems; the diploid Aglaozonia-stage has a broad encrusting thallus, something like that of a Ralfsia and growing with the help of a marginal meristem. In the development of the Aglaozonia-stage the zygote gives rise to a short erect thread, attached to the substratum by rhizoids and later undergoing longitudinal division to form a more or less massive club-shaped upgrowth (Fig. 4, A, B, F, c) which not uncommonly bears an apical tuft of hairs. This column (foot of Church, 1898, p. 86) remains short, and meristematic activity is soon restricted to its base and results in the formation of the Aglaozonia-crust



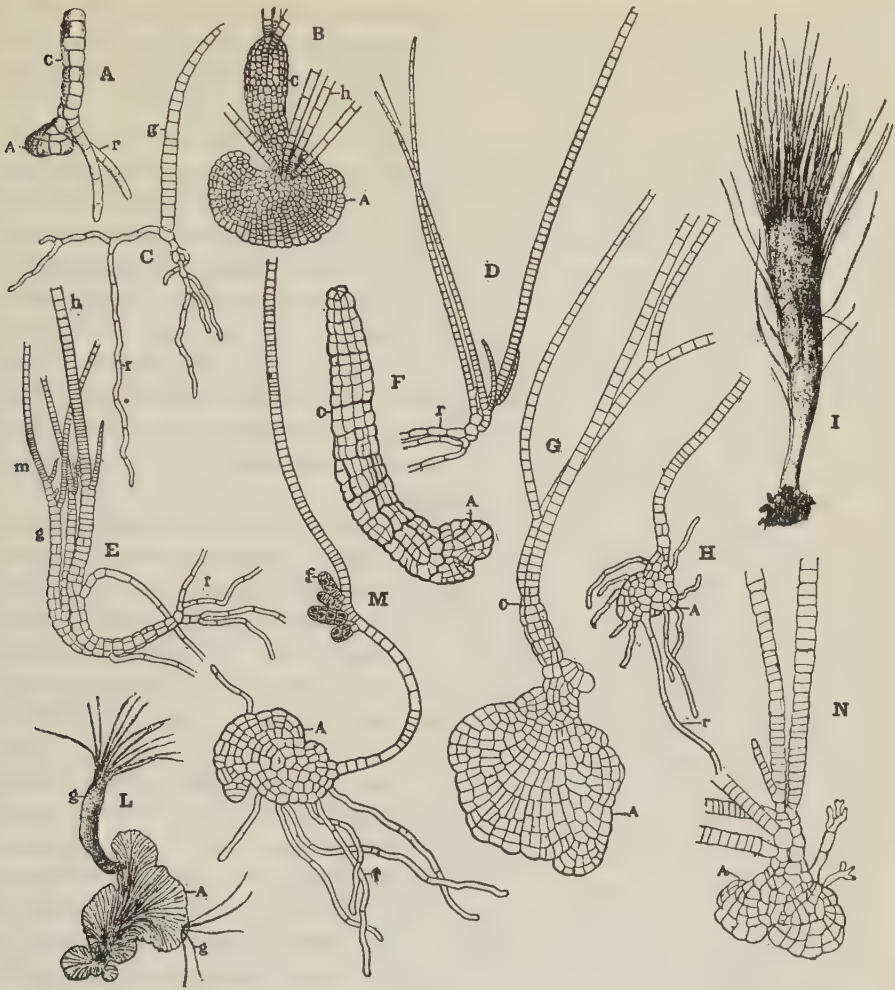


FIG. 4. Cutleria: B, N, *C. adspersa* (Roth) De Not.; L, *C. monoica* Oliv.; the rest *C. multifida* (Engl. Bot.) Grev. A, germling derived from zygote; B, F, older germlings derived from apogamous female cells; C-E, I, germlings of successive ages, derived from zoospores, in D only small part of rhizoid-system shown; G, H, N, forma Church; L, development of gametophytic thallus from Aglaozonia-stage by vegetative proliferation. A, Aglaozonia-crust; C, column; f, oogonia; g, gametophyte or gametophytic threads; h, hair; r, rhizoid. (A after Falkenberg; B, L, N after Sauvageau; F after Church; the rest after Kuckuck.)

(Fig. 4, A, B, F, A). The zoospores formed in the unilocular sporangia of the latter on germination likewise give rise to an erect thread (Fig. 4, c) attached by rhizoids (r), but here an intercalary meristem soon appears near the base of the thread and cuts off numerous segments on its lower side (Fig. 4, D). These produce successive branches which develop a similar trichothallic meristem and, as they multiply, come to lie in close apposition; later the

portions of the threads below the individual meristems coalesce to form a short compact cylinder (Fig. 4, E) which bears the free threads as an apical tuft (Fig. 4, I) and soon branches to produce the familiar thalli of *Cutleria multifida*.

Although a regular alternation between the two phases has been established in various localities (Falkenberg, 1879; Janczewski, 1883, p. 212; Yamanouchi, 1912), there are evidently many divergences from the normal, and these are not without interest from the point of view of the elucidation of the nature and origin of the two phases. Germination of the female cells without fertilization has frequently been recorded, but the products are very diverse, sometimes resembling young forms of *Cutleria* (Thuret, 1850, p. 242), sometimes resembling those of *Aglaozonia* (Church, 1898, p. 85; Sauvageau, 1899, pp. 335, 350), and sometimes being of an intermediate (Sauvageau, 1899, p. 330; forma Church, see below) or rudimentary character. There is at present no evidence to suggest that these stages obtained from parthenogenetic ova are ever anything else than haploid.

A similar diversity may be shown by the germlings arising from zoospores. Church (1898, p. 90) found that many of those produced by the zoospores of *C. multifida* developed into branched upright filaments, longitudinally divided in their lower part and bearing antheridia; such purely filamentous stages had already been encountered by Kuckuck (1894, p. 251) and described by him as var. *confervoides*. In some of Church's filamentous stages the lower part broadened into a small *Aglaozonia*-crust (Church, 1898, p. 91), and such germlings are usually described as the forma Church (Sauvageau, 1889, p. 329; cf. Fig. 4, N). Similar plants have been recorded by Kuckuck (1899b; cf. Fig. 4, G, H), some of them bearing oogonia (Fig. 4, M). The ordinary filamentous growths obtained by Church and others from zoospores are likely to be cultural stages of the normal dioecious gametophyte; they recall the precociously fertile juvenile stages that have been met with in the sporophytes of diverse Ectocarpales under conditions of culture. As pointed out above, the parthenogenetic ova produce not only fresh gametophytes, but seemingly rather more frequently *Aglaozonia*-stages, which are probably haploid and, when they mature, will form haploid zoospores. It is probably these zoospores that largely furnish the peculiar intermediate stages that are known as the forma Church.

It would thus seem that, side by side with the normal alternation, there is in many regions an extensive apogamous development of the female cells which results for the most part in the production of haploid *Aglaozonia*-individuals. In diverse localities, in fact, it would appear as though the majority of these stages may be haploid, and that these possibly propagate indefinitely by haploid zoospores. At the same time, however, the female cells and the swarmers of the haploid *Aglaozonias* can evidently also give rise to gametophytic stages. The probable existence of haploid *Aglaozonias* in nature, presumably originating in the first place by apogamous development

of female cells, has been strengthened by Sauvageau's (1931, p. 21) observations on the species *Cutleria monoica*. An apogamously produced plant of *Aglaozonia chilosa*, the corresponding asexual phase, was observed to give rise by vegetative proliferation to new *Cutleria*-plants (Fig. 4, L). How far this may be a widely distributed phenomenon remains to be seen.

The fact that in the forma Church an *Aglaozonia*-crust appears as a basal outgrowth from a gametophytic filament, often after the latter has undergone longitudinal septation in its lower part (Fig. 4, G), suggests that the column of the normal *Aglaozonia*-stage is to be regarded as an arrested and sterile structure, morphologically homologous with the *Cutleria*-thallus. Sauvageau (1899, p. 330) has, in fact, pointed out that in this form the *Cutleria*-threads are often borne at the tip of an evident column. The normal *Aglaozonia*-stage shows clearly its derivation from a heterotrichous ancestry, the column representing the erect system, the crust the prostrate system. The fact that the latter is here formed secondarily does not appear to be of much significance. The factors that have resulted in the dominance of the erect system in the *Cutleria*-stage may lead also in *Aglaozonia* to the primary formation of the upright column. Sauvageau's observations on the vegetative origin of new *Cutleria*-plants from the *Aglaozonia*-stage of *C. monoica* illustrates the presence of both parts of the heterotrichous system in what is to all intents and purposes one individual. This possibly marks a semblance of the original state of affairs when the alternation in *Cutleria* was isomorphic between two individuals with a heterotrichous habit, neither system of which then necessarily showed the specialization seen in the present-day forms. The sexual individual has lost the prostrate and the asexual the upright system, except for the relic preserved in the shape of the column. The *Aglaozonia*-stage is comparable to the fertile juvenile stages of the sporophyte of other Phaeophyceae and Rhodophyceae discussed above.

Such an interpretation accounts for the existence of a closely allied genus (*Zanardinia*) with purely isomorphic alternation and also explains the frequent association of gametophytic threads bearing sex organs with a prostrate *Aglaozonia*-crust (Fig. 4, M) in the same individual (the forma Church). Such an association is evidently not confined to the precociously fertile gametophytic stages, since according to Gran (1893, p. 25) *Aglaozonia*-stages can arise from young *Cutlerias* in which definite tissue-formation has already taken place.

#### SUMMARY AND GENERAL CONCLUSIONS

Heterotrichous forms are widely distributed in practically all classes of Algae that have attained a higher differentiation, being found in Chaetophorales (Chlorophyceae), Ectocarpales, Tilopteridales, and Sphacelariales (Phaeophyceae), Bangiales and Nemalionales (Rhodophyceae), Heterotrichales (Xanthophyceae), Chrysotrichales (Chrysophyceae), Pleurocapsales and



Stigonematales (Myxophyceae). The various modes of development of the heterotrichous habit (heterotrichous filament, prostrate filamentous and discoid types, crusts) are exhibited by parallel forms in the majority of these classes. In the more highly specialized members of Chaetophorales, Phaeophyceae, and Florideae the prostrate system is eliminated and growth is erect from the first; in the Phaeophyceae with heteromorphic alternation (Sporochnales, Desmarestiales, Laminariales) the gametophyte, however, retains its heterotrichy, while among Florideae stages intermediate between the heterotrichy of the less specialized types and its complete disappearance in Ceramiales are found among Cryptonemiales, Gigartinales, and Rhodymeniales.

All investigated Ectocarpales and Nemalionales pass through a primary heterotrichous stage; the same is true of diverse Sphacelariales, Cryptonemiales, and Gigartinales. In all such instances the mature thallus is built up from an erect filament or filaments of the juvenile stage. It is not uncommon (see p. 403) for the plant to remain arrested for considerable periods at this primary stage and, when this is so, the latter may bear asexual reproductive organs subserving its propagation (Kuckuck's prospory, 1899*a*; 1917). The filamentous stages (plethysmothalli) derived from the plurilocular sporangia of the sporophyte in Ectocarpales are no doubt of this nature; they are arrested sporophytes and sooner or later their erect filaments produce the macroscopic plant. Evidence is produced for the view that these stages are always diploid, while the filamentous growths (gametophytes) derived from the unilocular sporangia of the sporophyte are no doubt normally haploid (see article II). Sauvageau's distinction between plethysmothalli, gametophytes, and protonemata is misleading.

Heterotrichy is thus not only of widespread occurrence, but underlies the construction of all the less specialized members of Phaeophyceae and Rhodophyceae. It represents a stage reached in the evolution of all algal classes that have passed beyond the condition of the simple filament; from it originated the advanced types of thalli, although in the more specialized forms of the latter the prostrate system has been largely suppressed.

Cutleria, which is unique among the Algae in showing an alternation of two different macroscopic phases, the one growing erect, the other largely prostrate, shows in the intermediate forms that have come to light (see p. 408) distinct evidence of origin from an isomorphic ancestry which was heterotrichous. The sexual generation has lost the prostrate, the asexual the erect system, although traces of the latter persist in the shape of the column which initiates the Aglaozonia-stage.

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# An Investigation of Physiological Methods of Determining Nutrient Deficiencies in Sugar-cane

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With nine Figures in the Text

## INTRODUCTION

THE investigation of several possible methods of diagnosing mineral deficiencies in sugar-cane has recently been undertaken in the Sugarcane Research Station. Of the methods used, the leaf-diagnosis method of Lagatu and Maume has been investigated in all its aspects, chiefly in the Chemical Division. In addition, the application of the technique to various field trials, particularly those on the time of application of fertilizers, were undertaken by the Botanical Division. This method has been shown not only to be a reliable comparative method but, with certain safeguards, to have a high absolute value.

Three other possible methods were investigated in the Botanical Division, viz. the two methods which are described in this paper, and the chemical analysis of exudate from cut stems. The latter method, although capable of giving indications of deficiency where marked deficiencies occurred, was affected by numerous uncontrollable factors, often resulting in considerable difficulty in interpreting the results. For these reasons it was rejected as a possible method for fuller investigation. This report is confined to the examination of leaf and stem injection methods (Part I) and of the growth increment method (Part II, p. 17).

## PART I. RESULTS OF INJECTION EXPERIMENTS FOR THE DIAGNOSIS OF NUTRIENT DEFICIENCIES IN SUGAR-CANE

### I. *Micro-injections (leaf-injections)*

#### *Methods.*

An attempt has been made to apply the injection methods developed by Roach (1939) to the diagnosis of nutrient deficiencies in sugar-cane. The movement of injected liquids was followed by the use of dyes, acid fuchsin, methylene blue, and fluoresceine being used, the distribution of the latter was determined by viewing in ultraviolet light after treatment with ammonia gas, under which conditions brilliant fluorescence occurs.

In order to explain the type of injection obtained by different methods, the venation of the leaf must be considered. The venation of the sugar-cane leaf is not strictly parallel, the vascular strands joining with the midrib at intervals along its length (Fig. 1). The lamina is traversed by approximately 12–15 main vascular strands, between which is a very large number of smaller strands; all these are independent of each other along their whole length, i.e. there are no cross-connexions.

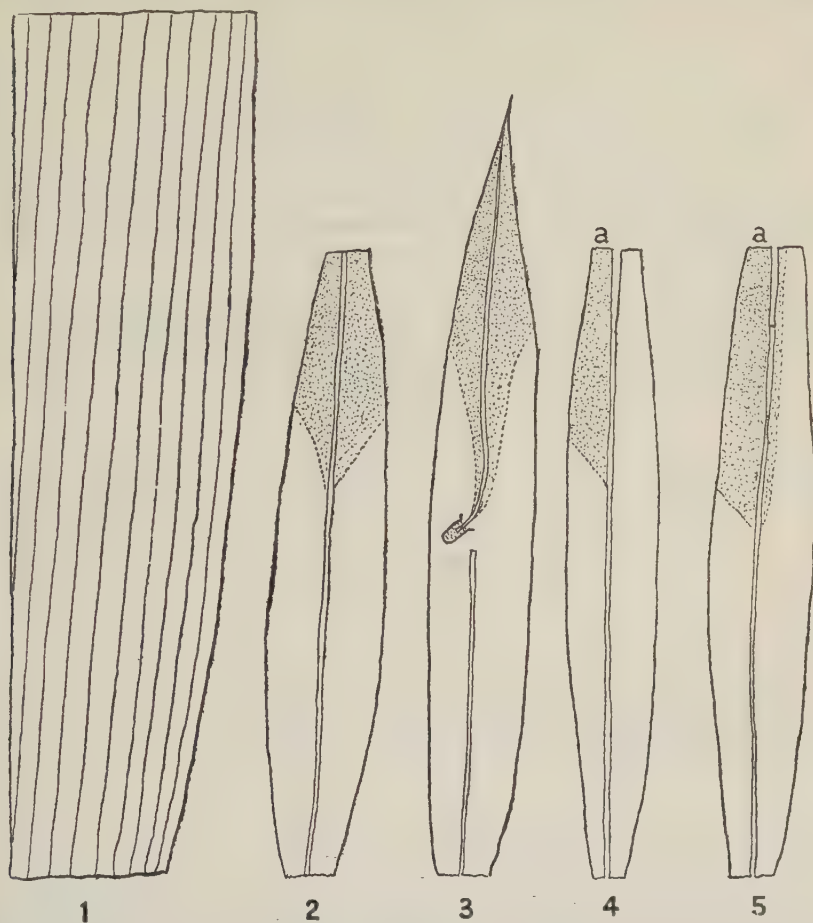
Roach's apparatus for interveinal injection of small leaves—capillary tube with cotton running through the tube and fixed to the base with paraffin wax (Roach, 1938)—resulted in a thin line of injected fluid above and below the puncture, there being no lateral spread of fluid. In order to obtain a broader injected band, small bottles were used with filter-paper wick replacing the cotton, these bottles being prepared by blowing a bulb in glass tubing; the curled filter-paper wick holds the bulbs in position. Using this method satisfactory injections were obtained by the use of dyes, a band equivalent in width to the width of the wick being injected with the dye. It was observed, however, that with this method there was a pronounced yellowing of the band above the cut when water alone was used for injection; this was undoubtedly due to the interruption in the supply of water caused by the severing of the vessels in making the incision. Neither of the two methods described above was therefore used, and new methods in which there were no such disturbing effects were employed. The following three methods gave a satisfactory injection of a considerable part of the leaf.

1. Leaf-tip injection, as used by Roach for the injection of long narrow leaves like peach leaves. The type of injection obtained by this method is shown in Fig. 2.
2. Midrib injection. The midrib on the lower surface of the leaf is slit for a certain distance and the portion thus freed placed in the liquid. The type of injection obtained is shown in Fig. 3.
3. The tip of the leaf is removed and the leaf then cut vertically on each side of the midrib for about 3 in., this portion of the midrib being removed; one half of the lamina is placed in the solution in a waxed paper bag and held on by a paper clip. Very good injection is obtained by this method, the area injected being partly dependent on the length of time during which injection proceeds. If the leaf is allowed to absorb for too long, the liquid passes into the midrib and from thence back along the 'control' part of the leaf, but injection may safely proceed for a period up to seven hours without danger of the fluid passing into the half of the leaf on the other side of the midrib (Figs. 4 and 5).

All these methods were tried with solutions of salts, the methods depicted in Figs. 2 and 3 being mainly used for young leaves only partly unfolded, the method depicted in Fig. 4 being the most useful for all other leaves. Even this method, however, has one considerable disadvantage, in that the two halves of a sugar-cane leaf are, particularly in young leaves, not comparable,



since, as the leaf unfolds, one half of the leaf (i.e. the half which unfolds first) has a distinctly deeper green colour than the half which unfolds later, and this difference persists for a considerable time afterwards. It is possible,



FIGS. 1-5. Fig. 1. Diagrammatic representation of the venation of a sugar-cane leaf, only major veins shown. Fig. 2. Result of leaf-tip injection (shaded area shows spread of dye). Fig. 3. Result of midrib injection. Fig. 4. Result of injection of half-leaf tip when injection proceeds for less than seven hours. Injected portion of leaf denoted by *a*. Fig. 5. Similar to Fig. 4 except that injection was allowed to proceed for one to three days.

however, to compare the injected and non-injected portions on the same side of the midrib.

#### *Experimental material.*

The experimental material was located in three main districts.

1. Plots not supplied with potash, on land known to be markedly deficient in potash at Cascade, Rose Belle, S.E.

2. Plots not supplied with phosphate at Bon Accueil in a region in which marked phosphate deficiencies had been proved to exist. These plots were placed at my disposal by the Senior Chemist of the Sugarcane Research Station. Large responses to potash and phosphate respectively had been obtained when these substances were added to the soil.
3. Plots at Reduit in which deficiencies in potash, nitrogen, and phosphate had been caused artificially by the removal of the surface soil over the whole plots to a depth of 8-9 in. The treatments in these plots were as follows: N+P+K, P+K, N+P, N+K, and no fertilizer. The differences in growth were quite remarkable; the N+P+K plot gave good normal growth, the K deficient plot was quite definitely poorer; the N deficient and the P deficient plots were extremely poor, the latter showing almost as poor growth as the no fertilizer plot.

When the plants in these plots were six months old leaf samples (3rd leaf) were taken, and the data of these leaves give some indication of the differences between the various treatments which existed at this time.

Plot treatment.	Mean wt. of leaf (gm.)	Mean length of leaf (cm.)	Mean breadth of leaf (cm.)	Moisture (per cent. fresh wt.)	N (per cent. of dry matter)	P <sub>2</sub> O <sub>5</sub> (per cent. of dry matter)	K <sub>2</sub> O (per cent. of dry matter)
O	6.3	94	3.0	76.1	1.24	0.24	1.48
N+K	7.2	96	3.2	76.8	1.48	0.19	1.88
P+K	7.8	103	3.2	77.9	1.26	0.27	1.90
N+P	13.0	115	4.4	78.3	1.60	0.27	0.68
Complete	16.7	127	5.0	79.4	1.56	0.26	1.92

There is, therefore, little doubt that the plots used were deficient in nitrogen, phosphate, and potash, though the deficiency of the latter was not so serious as that of the former two elements, judging from the growth of the plants.

### *Results.*

A number of injections were carried out with 0.05-0.1 per cent. solutions of K<sub>2</sub>SO<sub>4</sub>, KNO<sub>3</sub> and KH<sub>2</sub>PO<sub>4</sub> at Cascade, with no apparent response in any instance. Similarly injections of similar concentrations of KH<sub>2</sub>PO<sub>4</sub> and NaH<sub>2</sub>PO<sub>4</sub> at Bon Accueil failed to show any response. The greatest number of injections were made on the plots at Reduit. In a series carried out on 6/9/40 the following substances were injected:

- (a) N deficient plants, 0.05 per cent. urea.
- (b) K     ,,     ,,     0.05 per cent. potassium sulphate.
- (c) P     ,,     ,,     0.05 per cent. sodium dihydrogen phosphate.
- (d) To the N, P, and K deficient plants, a solution consisting of equal volumes of the above three solutions.
- (e) Two control plants injected with methylene blue to determine the extent of injection.

Absorption was allowed to continue for three days: there was penetration of

the dye to over half the length of the leaf and it encroached on the uninjected side of the midrib. All the treatments were carefully examined by direct and reflected light on 11/9/40, 19/9/40, 24/9/40, and 6/10/40. At no time was the slightest response perceptible in any of the treatments.

A similar series was carried out with the same solutions on 10/9/40, the injection being, however, allowed to continue for one hour. The methylene blue showed that all the veins, both the major and minor, were injected for a distance of about 9 in. from the tip. The injected leaves were examined on 19/9/40, 24/9/40, and 6/10/40; no responses were apparent.

Following these preliminary results, it was considered that the plots might have been deficient in elements other than those which had been withheld. Injections were, therefore, made with the range of compounds given below:

- A. Urea (0.01 per cent.).
- B. Potassium sulphate (0.01 per cent.).
- C. Disodium hydrogen phosphate (0.01 per cent.).
- D. Calcium chloride (0.01 per cent.).
- E. Magnesium chloride (0.01 per cent.).
- F. Manganese sulphate (0.005 per cent.).
- G. Boric acid (0.001 per cent.).
- H. Zinc sulphate (0.001 per cent.).
- I. Copper sulphate (0.001 per cent.).
- J. Ammonium molybdate (0.001 per cent.).
- K. Ferric chloride (0.001 per cent.).
- L. Complete solution containing 10 c.c. each of A, B, C, D, and E; 5 c.c. of F, and 1 c.c. of G, H, I, J, and K.

Injection was carried out on the second leaf from the spindle—this being the youngest fully developed leaf—and allowed to continue for three hours; controls with methylene blue and acid fuchsin showed penetration of the dye in all the veins for a distance of 9–10 in., and no encroachment on the uninjected side of the midrib. Apart from a toxic effect of the copper sulphate which caused a burning of the injected half of the leaf for about 6 in. from the cut end, there was no response whatsoever to the elements either singly or in combination (L). They were examined at three-day intervals for over three weeks.

Similar injection experiments using the above and also stronger solutions (0.05–0.1 per cent.) were carried out, using the methods shown in Figs. 2 and 3, on leaves which were only partly unfolded and in which the development of chlorophyll was not yet complete, and also on older leaves using the method shown in Fig. 4. No responses were obtained in any instance.

In these experiments movement of the dyes and the toxic effect of copper sulphate appears to show that there is no difficulty in the injection of substances into sugar-cane leaves; conclusive evidence on this point is supplied by the analytical results given later.

Even in cases where marked deficiency exists, as shown by the remarkable differences in growth in the differently treated plots, no response to the injection of the deficient element into the leaves has been demonstrated.

The marked deficiencies in these plots were further confirmed by treating some plants of each plot with the element which was lacking, by adding to the soil a dressing of the substance concerned. In three weeks there was a spectacular recovery of the treated plants, the recovery being quickest in the N deficient plants treated with sulphate of ammonia, where the leaves became dark green in colour and growth was rapidly resumed. In the K deficient plants treated with potassium sulphate all signs of potash deficiency disappeared and growth continued. In the P deficient plants treated with phosphatic guano the leaves became slightly deeper in colour, but the most notable result was the development of numerous new tillers.

Following these results, stem injections of the deficient plants with 0.5 per cent. solutions of the following substances were made:

N deficient.	K deficient.	P deficient.
Urea	Potassium sulphate	Sodium phosphate
Ammonium nitrate	Potassium chloride	

These injections were effected on every cane in the stool, the total volume of solution absorbed being 100–50 c.c. No change in colour of the leaves resulted, the leaves of the injected plants being quite indistinguishable from control leaves.

These results conclusively demonstrated the failure of the injection methods tried to produce any response in plants which were, in fact, markedly deficient in the elements injected; these deficiencies, on the other hand, were quickly made good by adding the deficient substances to the soil.

## II. *Stem Injections*

Preliminary experiments were carried out to determine the quantities of the various substances which could be introduced without damage. The method used was to bore a hole  $\frac{7}{8}$ th of the diameter of a growing cane, and to insert a glass tube bent into an L shape, with a large bulb blown in the top in which a hole is punctured, the purpose of the bulb being to increase the holding capacity. The tube and bulb held about 50 c.c. of solution.

Nitrogen was introduced into the cane in the form of urea in concentrations of 5, 10, 25, and 50 per cent. In all cases the whole of the 50 c.c. was absorbed in twenty-four hours, the 25 per cent. and 50 per cent. solutions resulted in a rapid browning and death of all the leaves, the lower concentrations causing no damage. Potash was introduced as  $K_2SO_4$  in a 5 per cent. and 10 per cent. solution. The 10 per cent. solution caused a slight yellowing of the leaves which was only temporary, the leaves subsequently recovering their green colour.

Phosphate was introduced as  $NaH_2PO_4$  in concentrations of 2 per cent. and 10 per cent. Slight damage was caused by the 10 per cent. solution,



reddish streaks appearing in the leaves after some days. These preliminary experiments showed (a) that the substances rapidly penetrated to the leaves (b) that when no more than 50 c.c. was absorbed concentrations below 10 per cent. did not cause damage.

The growth of four series of ten canes, each treated with the following substances, was determined at Reduit:

- I. Complete fertilizer, full strength (5% urea + 3%  $K_2SO_4$  + 2%  $KH_2PO_4$ ).
- II. Complete fertilizer, half strength (2½% urea + 1½%  $K_2SO_4$  + 1%  $KH_2PO_4$ ).
- III. Water.
- IV. Controls (untreated).

The amount absorbed by individual canes varied from 40 to 70 c.c. No signs of any damage were apparent in any of the treatments, but there was considerable sprouting of buds in the canes injected with the full-strength fertilizer and to a lesser extent in the half strength, but not in the water or controls. The mean growth increments (cm.) during a period of one month following the injections are given below:

I.	II.	III.	IV.
16.8 ± 2.4	18.9 ± 2.2	17.7 ± 2.5	18.2 ± 2.4

There was thus no significant difference in the growth rate following the treatment.

Several series of similar experiments were then carried out in a highly laterized soil at Union Park, S.E., where it was expected that marked responses would be obtained. Injections of both solid salts and solutions of salts were made. The solids were placed in holes made with a cork-borer and subsequently tightly corked; preliminary determinations had shown that salts applied in this manner were quickly taken up by the cane. In each experiment there were five series of canes treated with urea,  $K_2SO_4$ ,  $NaH_2PO_4$ , and a complete fertilizer (urea (5 pts.),  $K_2SO_4$  (5 pts.) and  $NaH_2PO_4$  (1 pt.)). In the solid injections about 2.5–3 gm. of the dry salts were placed in each hole; in the liquid injections the canes absorbed approximately 40 c.c. of the 10 per cent. solutions. There were no signs of damage in any of the treated canes. The mean growth increments of the canes in the solid injection experiments were as follows:

Growth increment (cm.) after	Urea.	$K_2SO_4$	$NaH_2PO_4$	Complete.	Controls.
15 days	16.3 ± 0.9	14.6 ± 0.35	16.9 ± 1.1	16.1 ± 1.3	17.4 ± 1.1
40 "	28.6 ± 1.5	25.7 ± 1.1	31.8 ± 2.2	30.0 ± 2.6	33.8 ± 1.3
65 "	37.6 ± 2.1	37.1 ± 1.9	44.8 ± 2.4	39.1 ± 3.1	44.6 ± 1.9

The mean increments in the liquid injection experiment are given below:

Growth increment (cm.) after	Urea.	K <sub>2</sub> SO <sub>4</sub>	NaH <sub>2</sub> PO <sub>4</sub>	Complete.	Controls.
25 days	14.4 ± 1.3	15.4 ± 2.1	19.3 ± 1.1	15.7 ± 2.2	19.0 ± 1.7
50 "	29.0 ± 1.6	29.1 ± 6.0	33.1 ± 3.9	31.3 ± 4.3	34.3 ± 2.9

The conclusions which may be drawn from the injection experiments at Reduit and at Union Park, S.E., are, therefore, that in no case was there a significant increase in the growth rate following injection of mineral salts. Both the solid and liquid injections at Union Park, S.E. (which were carried out in different fields), appear to indicate further that the growth of the canes injected with urea and with potassium sulphate may be lower than that of the untreated canes, whereas growth of the canes injected with sodium phosphate and with the complete fertilizer was not significantly different from that of the untreated canes.

All these preliminary experiments were, however, subject to the criticism that it was not known with certainty that responses to any of the chief fertilizer elements would normally be obtained in these localities. Further experiments were, therefore, carried out in localities where serious deficiencies were known to exist. The trials laid down by the Senior Chemist at Bon Accueil and at Cascade, Rose Belle, were used for this purpose. At Bon Accueil the plots which had been given no phosphate were used for injections. The two varieties M. 140/31 and P.O.J. 2727 were injected with KH<sub>2</sub>PO<sub>4</sub> + K<sub>2</sub>SO<sub>4</sub>, the solid injection method being used. The growth increments are given below:

Growth increment at end of	M. 140/31			P.O.J. 2727		
	KH <sub>2</sub> PO <sub>4</sub>	K <sub>2</sub> SO <sub>4</sub>	Controls.	KH <sub>2</sub> PO <sub>4</sub>	K <sub>2</sub> SO <sub>4</sub>	Controls.
25 days	8.6 ± 1.2	4.9 ± 0.7	4.1 ± 0.4	9.8 ± 2.3	7.8 ± 0.7	9.2 ± 0.6

This experiment thus gave quite contradictory results, the variety M. 140/31 showing a significant response to phosphate, but not to potash; whereas the variety P.O.J. 2727 did not respond to either potash or phosphate.

At Cascade, Rose Belle, S.E., where very serious potash deficiency exists and where the canes in the no-potash plots showed marked deficiency symptoms, injections were made with potassium sulphate, potassium dihydrogen phosphate, and potassium nitrate. Approximately 3 gm. of the salts were introduced into each cane. The growth increments following the injections were as follows:

Growth increment at end of	K <sub>2</sub> SO <sub>4</sub>	KH <sub>2</sub> PO <sub>4</sub>	KNO <sub>3</sub>	Controls (untreated).
12 days	1.3 ± 0.37	2.9 ± 0.53	2.5 ± 0.73	2.7 ± 0.90
35 "	5.0	6.6	5.9	5.8

Here also there is no indication of increased growth following potash injection: while there is some evidence that the canes injected with potassium sulphate may have grown at a slower rate.

In view of the fact that with one exception (M. 140/31 injected with phosphate at Bon Accueil) there was no response to injection, it was considered desirable to check the injection method with the leaf-diagnosis method (analysis of leaves of a specified rank).

Accordingly, leaf samples (leaves of 3rd rank) were taken from the early Redit and Union Park experiments, and from the Bon Accueil and Cascade experiments. The results of the analysis of these leaves are given below:

Locality.	Date of injection.	Nature of injection.	Date of sampling.	Per cent. dry matter.		
				K <sub>2</sub> O	P <sub>2</sub> O <sub>5</sub>	N
Redit	3/2/39	Injected with complete fertilizer	22/3/39	2.24	0.262	1.21
"	"	Controls (untreated)	"	2.20	0.262	1.24
Union Park S.E.	12/3/39	Solid injection NaH <sub>2</sub> PO <sub>4</sub>	16/5/39	2.05	0.45	1.53
"	"	" K <sub>2</sub> SO <sub>4</sub>	"	2.11	0.37	1.41
"	"	" (complete fertilizer)	"	2.32	0.40	1.50
"	"	Solid injection (urea)	"	2.03	0.40	1.52
"	"	Controls (untreated)	"	2.09	0.42	1.44
Union Park	27/3/39	Liquid injection NaH <sub>2</sub> PO <sub>4</sub>	16/5/39	2.76	0.49	1.64
"	"	" K <sub>2</sub> SO <sub>4</sub>	"	2.94	0.48	1.79
"	"	" (complete fertilizer)	"	2.90	0.52	1.87
"	"	Liquid injection (urea)	"	2.78	0.45	1.76
"	"	Controls (untreated)	"	2.83	0.46	1.58
Bon Accueil	4/5/39	Solid injection KH <sub>2</sub> PO <sub>4</sub> M.140/31	8/5/39	2.12	1.09	No N applied, therefore not determined.
"	"	Solid injection K <sub>2</sub> SO <sub>4</sub> M.140/31	"	1.80	0.20	
"	"	Controls (M.140/31)	"	2.17	0.23	
Bon Accueil	4/5/39	Solid injection KH <sub>2</sub> PO <sub>4</sub> M.140/31	28/5/39	2.12	0.33	—
"	Second sample of above	Solid injection K <sub>2</sub> SO <sub>4</sub> M.140/31	"	2.22	0.16	—
"	"	Controls (M.140/31)	"	2.14	0.18	—
Bon Accueil	4/5/39	Solid injection KH <sub>2</sub> PO <sub>4</sub> P.O.J. 2727	8/5/39	2.08	0.88	—
"	"	Solid injection K <sub>2</sub> SO <sub>4</sub> P.O.J. 2727	"	2.09	0.24	—
"	"	Controls (P.O.J. 2727)	"	1.92	0.23	—
Bon Accueil	4/5/39	Solid injection KH <sub>2</sub> PO <sub>4</sub> P.O.J. 2727	28/5/39	2.64	0.37	—
"	Second sample of above	Solid injection K <sub>2</sub> SO <sub>4</sub> P.O.J. 2727	"	2.43	0.22	—
"	"	Controls (P.O.J. 2727)	"	2.28	0.22	—
Cascade	11/5/39	Injected with K <sub>2</sub> SO <sub>4</sub>	23/5/39	1.71	0.42	1.71
Rose Belle	"	" KH <sub>2</sub> PO <sub>4</sub>	"	1.75	0.60	1.70
"	"	" KNO <sub>3</sub>	"	2.17	0.40	1.63
"	"	Controls	"	1.39	0.48	1.64



These analyses revealed features of considerable importance. The analysis of leaves from the Reduit experiment and the two Union Park experiments showed no significant difference between the injected and control plants approximately seven weeks after the injection. For this reason, samples were taken from Bon Accueil very soon after the injection (four days), and a second sample was taken twenty-four days after the injection. In the samples taken four days after the injection, the phosphate content of the leaves of those plants which had been injected with  $\text{KH}_2\text{PO}_4$  had increased enormously (from 0.22 to 1.09 per cent. in M. 140/31, and from 0.23 to 0.88 per cent. in P.O.J. 2727). The surprising aspect of the results, however, was that there was no increase in the potash content of the leaves in canes injected with  $\text{K}_2\text{SO}_4$  or with  $\text{KH}_2\text{PO}_4$ . Since the phosphate which had entered into the leaves (as proved by the analysis) had been supplied as potassium phosphate, it was expected that the potash also would have entered the leaves, but four days after the injection there was no difference in the potash content of the leaves of the injected and control plants.

The second samples which were taken at Bon Accueil twenty-four days after the original injection showed a marked drop in phosphate content (from 1.09 to 0.33 in M. 140/31 and from 0.88 to 0.37 in P.O.J. 2727), though it was still considerably higher than the controls. The potash content also showed no increase in M. 140/31, but did show some increase in P.O.J. 2727, the increase being apparently somewhat greater in the plants injected with  $\text{KH}_2\text{PO}_4$  than in those injected with  $\text{K}_2\text{SO}_4$ .

Analysis of the leaf samples from Cascade (taken 12 days after the injection) showed an increase in the potash content of all the plants injected with potash salts over the untreated controls. The increase was greatest in those plants which were injected with potash in the form of potassium nitrate. The phosphate content of those plants treated with phosphate was also higher than those plants which were not injected with phosphate.

The nitrogen content of the plants injected with nitrogen (as  $\text{KNO}_3$ ) was identical with those which had not received nitrogen.

The results of these analyses thus showed that an increase in the content of injected substances in the leaves was a transient phenomenon. It was considered necessary to have data on the fate of the salts injected. For this purpose recourse was had to the use of acid fuchsin. A volume of 50 c.c. of a 1 per cent. solution of acid fuchsin was injected into each of four canes: one cane being split open at intervals of six hours and the course of movement of the dye followed. Penetration was extremely quick, it had traversed the vascular bundles up to the apex in six hours, and in twelve hours was seen as broad red bands in the leaves. The following day it was noticed that the leaves of canes which were not injected but which were in close proximity to the injected canes were spattered with drops of the red dye, and on examination it was apparent that the dye had been exuded from the margin of the leaves of the treated canes during the night. It appeared that exudation



had been particularly active through the margin of the upper third of the leaf.

In view of the results obtained with acid fuchsin, canes of the variety M. 134/32 were injected with a solution containing urea, potassium phosphate, and potassium nitrate, and boiling tubes were attached to each leaf to collect the exudate. No attempt was made to collect the exudate quantitatively, but in general 15-25 c.c. of exudate was obtained overnight. The exudate was shown to contain large quantities of potash, nitrogen, and phosphate even as early as eighteen hours after the injection was made.

When the solid injection method was used, however, only traces of the substances injected were found in the exudate the following day, and analysis of exudate collected for a further six days showed no more than traces of the substances injected.

At the end of one week all the leaves were stripped and analysed. The results of the analysis for the liquid injection, solid injection, and untreated controls are given below:

			Liquid injection.	Solid injection.	Controls.
N	.	.	1.74	1.76	1.61
P <sub>2</sub> O <sub>5</sub>	.	.	0.606	0.369	0.292
K <sub>2</sub> O	.	.	2.496	2.368	2.376

Whereas there is no evidence of a significant increase in potash and nitrogen, there is a marked increase in phosphate, the increase being considerably greater in the liquid injection.

A further more comprehensive experiment was carried out at Reduit, B.H. 10/12 canes in the no-phosphate plots of trial Bio. 5 being used. This trial had given considerable responses to phosphate. A complete mixture of equal parts by weight of urea, KH<sub>2</sub>PO<sub>4</sub>, and KNO<sub>3</sub> was used throughout the experiment. The following treatments were effected:

#### i. Solid Injection.

- A. Small quantities given every two days in holes 5 mm. in diameter; a different hole being used for each application (12 canes).
- B. Large quantity given in hole 1.3 cm. in diameter every three days, the application being made in the same hole (12 canes).
- C. Large quantity given once in a hole 1.3 cm. diameter (30 canes): this treatment was used for periodical analysis of leaves of 3rd rank.

#### ii. Liquid Injection.

- D. Dilute solution ( $\frac{1}{2}$  per cent.) of complete mixture given every two days, using different hole for each injection (12 canes).
- E. Strong solution (10 per cent.) of complete mixture given every five days, using different hole for each injection (12 canes).

iii. *Controls.*

F. With holes bored (12 canes).

G. Without holes (12 canes).

In treatment B the rind began to split near the hole after the fourth injection, and it was noticed that liquid was being exuded through the cracks. A new hole was, therefore, made for the subsequent injections: only two holes were made during the twenty-five days of injection. In treatments D and E all the liquid supplied (40–45 c.c.) was absorbed in the first injections, but the volume of liquid absorbed in subsequent injections was small, partly owing to unfavourable weather. The canes in treatment C were not measured, as this treatment was specially included for leaf analysis. The growth increments in the other treatments over a twenty-five-day period were as follows:

Treatments.	A.	B.	D.	E.	F.	G.
Growth increment in 25 days	7.6±0.57	9.7±0.69	8.9±0.70	11.3±1.1	6.5±0.71	7.9±0.90

There thus appears to have been a significantly greater growth increment in treatment B, which received several doses in the same hole, and in treatment E which received a strong solution in different holes than in the other treatments. The treatments which received smaller quantities or dilute solution were not significantly better than the controls.

Samples of six leaves of the 3rd rank were taken from treatment C at intervals of 1 day, 2 days, 5 days, 10 days, and 20 days after the injection; and controls were taken at the beginning of the experiment and at fourteen days. The results of the leaf analysis are given below.

Sample.	Control I.	C <sub>1</sub> 1 day.	C <sub>2</sub> 2 days.	C <sub>3</sub> 5 days.	C <sub>4</sub> 10 days.	Control II 14 days.	C <sub>5</sub> 19 days.	C <sub>6</sub> 27 days.
N	1.48	1.53	1.50	1.42	1.35	1.339	1.35	1.29
K <sub>2</sub> O	2.50	2.26	2.61	2.38	2.52	2.21	2.49	2.09
P <sub>2</sub> O <sub>5</sub>	0.29	0.34	0.40	0.41	0.28	0.25	0.29	0.32

These results are plotted in Fig. 6.

While there is no evidence of any marked change in the potash and nitrogen content of the 3rd leaves, the increase in phosphate content is clear and unmistakable. The increase occurs fairly soon after the injection. There appears to be a marked fall after five days (probably due to loss by exudation), followed by a slow and steady rise, probably due to the washing out of further amounts from the stem.

Leaves of the 3rd rank were also collected from treatments A, B, D, E, F, and G twenty-four days after the injection and analysed. The results of the analysis are given below:

Treatment.	A.	B.	D.	E.	F.	G.
N	1.53	1.55	1.39	1.76	1.42	1.45
K <sub>2</sub> O	2.42	2.42	2.42	2.78	2.43	2.35
P <sub>2</sub> O <sub>5</sub>	0.42	0.45	0.28	0.51	0.24	0.26

Considering the different methods of injection, it is apparent that the method which has resulted in the largest increases in the content of the substances injected in the 3rd leaf was that in which a strong (10 per cent. solution) was injected at intervals. This confirms the similar result obtained

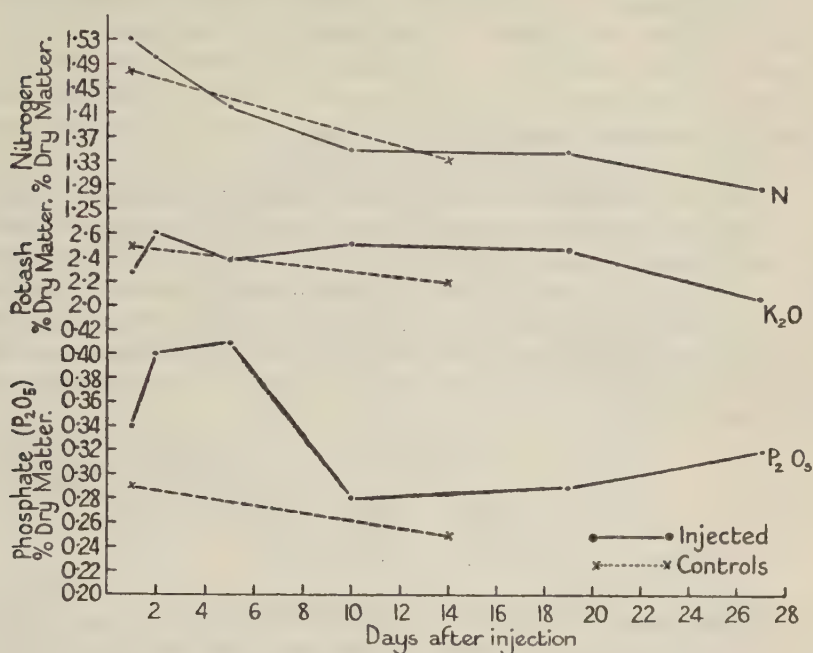


FIG. 6. Leaf analysis following injection.

in the experiment with M. 134/32. The solid injection method with one or two large holes filled with the fertilizer mixture at intervals was the second best. The injection of dilute ( $\frac{1}{2}$  per cent.) solution had no significant effect.

With respect to the increase in the content of the different elements all the methods of injection have caused an increase in the phosphate content over the controls, methods E, B, and A being responsible for the largest increases. Only method E caused an increase in potash, and the increase even with this method was small. Method E also resulted in the largest increase in nitrogen, although in the case of this element a small increase resulted from the use of methods A and B.

Considering the results obtained to this stage, it is apparent that where the phosphate content of the plant is rather low, a marked increase in phosphate content is obtained by any method of injection. In those instances where the phosphate content of the leaves indicates little or no phosphate deficiency, there is generally no increase in phosphate content, or at least only a small increase when phosphate is injected. Under conditions of phosphate deficiency the increase in phosphate content obtained by injection of phosphate,

often, though not invariably, results in an increased growth of the treated canes.

With regard to potash and nitrogen the situation is different. In all the preliminary experiments there was no increase in the potash content of the 3rd leaf irrespective of the method of injection, with the exception of the Cascade, Rose Belle, experiment. In this locality a marked potash deficiency was known to exist, and injections of potash in the form of  $K_2SO_4$ ,  $KH_2PO_4$ , and  $KNO_3$  all caused an increase in the potash content of the 3rd leaf as compared to untreated controls. There was, however, no corresponding increase in the growth rate following the injection.

Since, however, the only case of an increase in potash content was obtained in a locality where potash was known to be deficient, further experiments were carried out in the no-potash plots of trial Bio. 5 at Reduit. This locality also gave considerable responses to potash. In the first experiments, a complete mixture of equal parts by weight of  $KNO_3$ , urea, and  $KH_2PO_4$  was injected in the solid form. Analysis of leaves for  $K_2O$  and  $P_2O_5$  content was made four days and twenty days after the injection. The results of the analysis are given below:

		4 days after injection.		20 days after injection.	
		$K_2O$	$P_2O_5$	$K_2O$	$P_2O_5$
Treated	.	1.86	0.392	1.46	0.351
Controls	.	1.88	0.337	1.35	0.273

In this experiment no significant increase in potash content was obtained, although the increase in phosphate content was quite marked. A further injection was then made in the same canes, but this time using potassium sulphate only, in the dry powder state. At the same time a new series was started in which a 10 per cent. solution of potassium sulphate was injected.

Leaf samples (3rd leaves) from injected and control canes were taken seven days after the injection.

		$K_2O$ per cent. dry matter.	$P_2O_5$ per cent. dry matter.
Injected with solid $K_2SO_4$	.	1.016	0.280
Controls	.	0.888	0.294
Injected with liquid $K_2SO_4$ (10 per cent. solution)	.	1.988	0.276
Controls	.	1.484	0.255

In both cases increases in the potash content of the 3rd leaf occurred following injection, but the increase was greater in those injected with a 10 per cent. solution.

A similar experiment was carried out on the no-potash plots at Cascade, Rose Belle. There were two treatments, viz. liquid injection of 10 per cent. complete mixture (5 parts urea+5 parts  $KNO_3$ +5 parts  $KH_2PO_4$ ), solid



injection of powdered  $K_2SO_4$ , and untreated controls. The treatments were applied on 19/7/39, and the 1st-leaf samples taken fourteen days later.

	$K_2O$ per cent. dry matter.	$P_2O_5$ per cent. dry matter.
Injected with liquid (10 per cent. complete fertilizer) . . . . .	1.560	0.429
Injected with solid $K_2SO_4$ . . . . .	1.384	0.301
Controls . . . . .	0.992	0.298

Here also increases in the potash content of the leaves were obtained, and in the liquid injection series (which contained phosphate also) an increase in phosphate was also obtained.

The treated and control canes in all these experiments were measured. The growth increments over the specified time periods are given below.

Redit	Solid $K_2SO_4$ cm.	Controls.	Liquid $K_2SO_4$ (approx. 10 per cent.)	Controls.
Growth increments in 38 days	$10.33 \pm 0.65$	$10.91 \pm 0.72$	$12.59 \pm 0.73$	$10.75 \pm 1.00$
Differences in favour of treated Cascade, Rose Belle	$-0.58 \pm 0.97$	—	$+1.84 \pm 1.23$	—
	Liquid injection (10 per cent. complete).	Solid injection $K_2SO_4$	Controls.	
Increment in 33 days . . . . .	$4.07 \pm 0.90$	$2.8 \pm 0.5$	$2.0 \pm 0.6$	
Differences in favour of treated	$+2.07 \pm 1.08$	$+0.8 \pm 0.78$	—	

While none of these results is as marked as might be expected, there is a suggestive increase in the growth of the canes injected with liquid containing complete fertilizer over the controls.

During my absence on European leave two further experiments were carried out by my assistant, Mr. A. d'Emmerez de Charmoy, at Bon Accueil and Cascade, Rose Belle, phosphate and potash deficient lands respectively.

At Bon Accueil twenty canes of M. 140/31 were selected for injection and twenty canes as controls in the no-phosphate plots, and similar numbers in the plots supplied with phosphate. Sodium monohydrogen phosphate in 5.8 per cent. solution was injected into the treated canes, no dihydrogen phosphate being available. Each cane absorbed 50 c.c. of the solution. The canes were measured after twelve days and after twenty-seven days. The results of the growth measurements and the  $P_2O_5$  content expressed as per cent. dry matter were as follows:

	-P plots.		+P plots.	
	Injected.	Controls.	Injected.	Controls.
Growth increment after 12 days	5.2	5.9	10.5	13.0
" " after 27 days	10.3	12.0	18.3	24.8
$P_2O_5$ content (12 days after injection)	0.70	0.24	0.60	0.27

The difference in the growth rates of the canes in the  $-P$  and  $+P$  plots indicate the growth response to the phosphate dressing applied in the soil. In so far as response to injection is concerned, there appears to be a distinctly lower growth rate in the injected plants.

As this experiment only differs from the previous one carried out in this locality in the use of the monohydrogen phosphate instead of the dihydrogen phosphate, the decrease in the rate of growth (a fact which was not previously observed) may be due to the use of the former salt.

A similar experiment was carried out at Cascade, Rose Belle, but in this case bored and unbored canes were used as controls. In the treated canes 50 c.c. of 10 per cent. potassium sulphate was introduced into each cane. The mean growth increments after 10 days are given below in centimetres.

- K plots.			+ K plots.		
Injected.	Bored control.	Control.	Injected.	Bored control.	Control.
2.3	4.7	4.7	3.9	6.9	6.3

These measurements appear to confirm previous indications that the injection of potassium sulphate even under conditions of potash deficiency causes a reduction in the growth rate. The actual boring of the cane (as was shown previously at Redit) was not responsible for this lowered growth rate. The potash content of samples of the 3rd leaves taken ten days after the injections at Cascade are given below:

		K <sub>2</sub> O content per cent. dry matter.	
		- K plots.	+ K plots.
Controls .	.	0.78	1.72
Injected .	.	0.96	1.82

The slight increase in potash content, following injection, did not thus cause any increase in the growth rate.

## DISCUSSION AND SUMMARY OF PART I

It should be emphasized that even in cases where a reduction in the growth rate resulted from injection, there were no apparent symptoms of any damage to the leaves of the injected plants. On the other hand, where injections of elements known to be deficient actually resulted in considerable and maintained increases in the content of such elements in the leaf, there was no apparent improvement in the colour of the leaves, a fact which is in accord with the results of the micro-injection of elements into the leaf, in which no improvement in the colour of the injected leaves could be established.

It is apparent from the results presented that the factors involved in the injection of mineral salts into sugar-cane are complicated. It is a simple matter to increase the phosphate content of the leaves by stem injection, although the extent of the increase appears to be affected partly by the

amount supplied and partly by the previous phosphate content of the leaves. Leaves wherein the phosphate content was low appeared to show the greatest increases. Injection sometimes caused an increased growth rate, but often caused no increase in growth or even a considerable decrease. The same statement applies to potash. Increases in the potash content of the leaves following stem injection are more difficult to produce, and relatively large quantities of the salt have to be introduced before an increase in the potash content of the leaves can be demonstrated. Moreover, it was only in cases where potash deficiency was known to exist that increases in the potash content of the leaves following stem injection were obtained. No increase in the growth rate followed injection with potash salts even when deficiencies existed; there was, in fact, often a decrease in the growth rate. With respect to nitrogen, no increase in the nitrogen content of the leaves was produced by the injection of moderate quantities: even with high concentrations of nitrogen in the liquid injected, the increase in the nitrogen content of the 3rd leaf was small. In this case also no growth responses following injection could be established. Judging from the results obtained to date, it does not appear that leaf or stem injection offers much promise for the diagnosis of nutrient deficiencies in sugar-cane.

## PART II. THE USE OF GROWTH INCREMENT DATA TO DETERMINE NUTRIENT DEFICIENCIES

### *Preliminary Data*

In 1940 the Scientific Assistant of the Botanical Division measured the rate of growth of sugar-cane in the light and heavily fertilized plots of the Chemical Division at Reduit. These data, which are plotted in Fig. 7, established the fact that the canes in the heavily fertilized plots had a significantly higher growth rate than those in the low fertilized plots during the hot summer months, but that thereafter there was no significant difference between the growth rates. This result showed that most of the response to fertilizer occurred during the period of active growth when climatic factors, particularly soil moisture and temperature, were either not limiting or were limiting to a lesser extent than in the cooler months. It is thus apparent that with these high levels of climatic growth factors, the supplies of essential mineral substances were inadequate for full growth in the low fertilizer plots.

As a corollary, the conclusion may also be drawn that the response measured during the period of active growth would be considerably greater than the average effects over the whole season as measured by yield differences at harvest. In these preliminary growth measurements forty canes selected at random in each treatment were measured, so that the responses were obtained in terms of the growth increments of individual canes. It is a well-known fact, however, that fertilizers may not only increase the growth rate of individual canes but may also cause increased tillering. To obtain the

maximum growth increment would thus entail the measurement of whole stools.

### Uniformity trials.

In order to obtain statistical data on the growth of sugar-cane stools in the

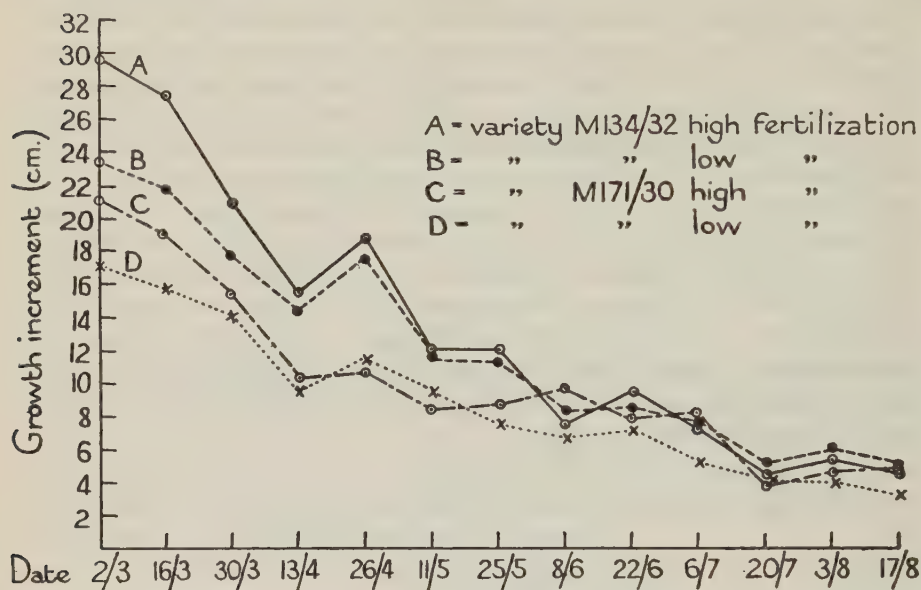


FIG. 7. Growth curves of two varieties under high and low fertilization.

field, measurements were made on several uniformity trials each consisting of twenty-five stools on which a  $5 \times 5$  latin-square design could be superimposed. These uniformity trials consisted of virgin or plant canes from four to twelve months old and several categories of ratoon canes.

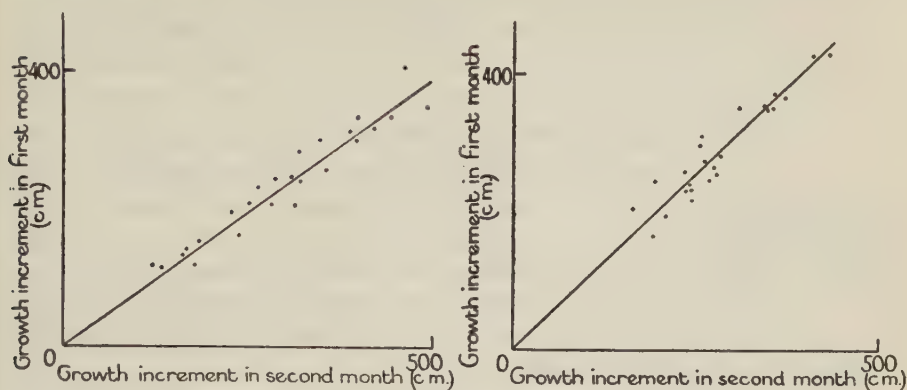
As was expected from previous observations, the variation in the total length of cane per stool was considerable, the significant difference averaging 42 per cent. of the mean length of cane per stool. The stools were again measured after an interval of one month so that the monthly growth increment could be calculated. The growth data are given in Table I.

TABLE I

	Reduit 1.	Reduit 2.	St. Aubin.	Beau Champ.	Planter Flacq.	Planter Phoenix.	Union Park.
Mean growth increment during 1st month (cm.)	258.9	281.4	281.7	157.7	163.1	190.0	115.1
S.E. of diff. between two means	53.5	63.0	46.6	32.1	28.4	19.8	26.2
Sig. diff. $P=0.05$	112.5	132.4	98.8	68.1	60.2	43.1	55.5
Sig. diff. per cent. mean	43.4	47.1	35.1	43.1	36.9	22.7	48.2



The variations in the growth increments of the stools are of the same magnitude as those of the original stool lengths. These variations are, in fact, largely due to the original variations in the size of the stools, and their magnitude are such that a large number of stools would have to be measured to establish the expected differences in the growth of the stools as a result of



FIGS. 8 and 9. Fig. 8. Uniformity trial, Reduit 1. Fig. 9. Uniformity trial, Reduit 2.

fertilizer applications. However, the fact that the variation found is due, in large measure, to the original size of stool results in a considerable reduction in the magnitude of the variation when the effect of size of stool is corrected for. This can be done in several ways, the most satisfactory of which is by the analysis of covariance. In the present problem, however, the important consideration is the consistency in behaviour with respect to growth between one period and another succeeding period. In order to determine this degree of consistency in growth behaviour the growth increments of the stools for a second month were determined. When the growth increments of the stools during the first month were plotted against the corresponding values during the second month, a very high degree of correlation is apparent. The curves for the uniformity trials Reduit 1 and Reduit 2 are shown in Figs. 8 and 9.

The trial Reduit 1 consisted of adjacent stools in adjacent rows, while the trial Reduit 2 consisted of alternate stools in alternate rows. The correlation coefficients between the growth increments in the two months were  $+0.927 \pm 0.0994$  for trial Reduit 1 and  $+0.885 \pm 0.1531$  for trial Reduit 2.

The method evolved for determining nutrient deficiencies by means of growth increments is based on this high correlation between the growth increment of stools in a preliminary period and that in a subsequent 'experimental' period.

#### *Method adopted.*

In the technique finally adopted twenty-five stools (five rows of five alternate stools), on which a latin-square design could be imposed, were measured. The growth increment of these stools was determined over

a period of one month, before any treatments were applied. At the end of this period a latin-square design selected by randomization was imposed and the following treatments applied:

- O. Controls, no fertilizer given.
- N. A dressing of sulphate of ammonia at the rate of 300 lb. per acre.
- P. A dressing of precipitated phosphate at the rate of 300 lb. per acre.
- K. A dressing of sulphate of potash at the rate of 150 lb. per acre.
- NPK. A dressing of sulphate of ammonia, precipitated phosphate, and sulphate of ammonia at the rates given above.

Adequate care was taken that the fertilizers were efficiently applied so as to become quickly available. The method of application used was as follows. The superficial soil in a circle of about 1 ft. radius around the stool was carefully removed until a dense matting of roots appeared and holes 6–8 in. deep were made around the stool with a small crowbar. Some of the fertilizer dressing was placed in these holes and the remainder broadcast over the exposed roots and covered with soil.

#### *Rate of absorption of salts applied to the soil.*

An experiment was carried out to obtain some idea of the time which elapsed between the application of salts to the soil by the method described above and their appearance in the 2nd, 3rd, and 4th leaves from the apex (the seat of active leaf metabolism). Stools in the uniformity trial Reduit 1 were given a dressing of potassium bromide at the rate of 30 gm. per stool. This substance was used, since: (1) sugar-cane leaves do not normally contain bromide, (2) it is not injurious to the plant, and (3) it is easily detected.

Leaf samples were taken 24, 40, and 72 hours after application to the soil and leaf samples from untreated plants were taken at the same time. A positive bromide reaction was given in the case of the treated plants after 24 hours, and after 40 and 72 hours the reaction was very strong. In no instance was the slightest positive reaction given by the control leaves. It is thus apparent that salts applied to the soil are, under favourable conditions, located in the leaves wherein active metabolism is proceeding within a day or two of their application.

#### *Growth results.*

The growth increments during a period of one month following the application of the treatments were determined. The figures which are analysed are the differences between the monthly growth increments before the treatments were applied and those in the month following the treatments. The method of analysis is illustrated for the trial on a small Indian planter's land in Flacq. The material consisted of 12-month-old canes of M. 27/16 which had made comparatively poor growth.

Monthly growth increment before treatment.					Monthly growth increment after treatment.					Differences between preliminary growth increments and those following treatments.				
P	N	K	O	NPK	N	K	O	NPK		P	N	K	O	NPK
148	154	97	184	154	223	334	220	265	459	+75	+80	+123	+81	+305
O	P	N	NPK	K	O	P	N	NPK	K	O	P	N	NPK	K
160	60	194	134	134	152	197	314	466	257	-8	+117	+120	+332	+123
K	NPK	O	P	N	K	NPK	O	P	N	K	NPK	O	P	N
160	102	149	207	154	250	273	235	318	242	+90	+171	+86	+111	+107
N	O	NPK	K	P	N	O	NPK	K	P	N	O	NPK	K	P
224	203	173	153	119	409	249	374	228	163	+185	+46	+201	+75	+44
NPK	K	P	N	O	NPK	K	P	N	O	NPK	K	P	N	O
201	275	229	170	119	491	360	250	292	158	+290	+85	+21	+122	+39

The differences between the increments in the preliminary period and those in the period following treatments are analysed by Fisher's analysis of variance. The result of this analysis is given below:

	O.	N.	P.	K.	NPK.	General mean.	S.E. of difference bet. two means.	Significant difference.
Mean differences between 'preliminary' and 'experimental' growth increments . . .	+48.8	+122.8	+73.6	+99.2	+259.8	+120.8	32.2	70.16
Differences between treatments and controls . . .	0	+74.0	+24.8	+50.4	+211.0	—	—	70.16

The application of the individual treatments produced an increase in growth over the controls, but of these only the nitrogen and the NPK effects were significant. The large response to the NPK treatment, however, suggests a considerable interaction effect, i.e. individual elements while not producing a significant effect alone, caused a very marked effect when combined with others.

### The Redit experiments.

The growth increments of the two uniformity trials at Redit were determined over a second month but received no treatments prior to the second month. A latin-square design was imposed on the results, the analysis of which is given below:

		Hypothetical treatments.					S.E. of difference between two means.	Significant difference.	
<i>Trial Reduit 1</i>		O.	N.	P.	K.	NPK.			General mean.
Differences in the growth increments between the first and second months .		+21.0	+73.6	+45.8	+28.6	+22.2	+38.2	28.6	62.3
Differences between 'hypothetical treatments' and controls		0	+52.6	+24.8	+7.6	+1.2	—	—	62.3
<i>Trial Reduit 2</i>									
Differences in the growth increments between the first and second months .		+21.3	—8.8	—8.2	—7.4	+3.2	+0.1	37.7	82.1
Differences between 'hypothetical treatments' and controls .		0	—29.5	—30.1	—28.7	—18.1	—	—	82.1

In both trials, as would be expected, none of the observed differences between the 'hypothetical treatments' and the controls was significant.

In the case of trial Reduit 2, the treatments O, N, P, K, and NPK were applied after the two preliminary determinations of monthly growth increments, and the growth increments for a period of one month following the application of the treatments, determined. The results were as follows:

	O.	N.	P.	K.	NPK.	General mean.	S.E. of difference between two means.	Significant difference.
Differences in the growth increments between the 'preliminary' period and the period following treatments . . .	+23.4	+44.0	+57.0	+37.8	+71.8	+46.8	14.9	32.5
Differences between treatments and controls	—	+20.6	+33.6	+14.4	+48.4	—	—	32.5

The phosphate alone and the NPK treatment produced significant growth responses. There is no evidence of any interaction, there being, apparently, only a slight phosphate deficiency.

The results of the trials carried out in other localities are given below:

*St. Aubin*

	O.	N.	P.	K.	NPK.	General mean.	S.E. of difference between two means.	Significant difference.
Differences in growth increments between preliminary and treatment periods . . .	+98.2	+131.6	+117.6	+108.0	+141.8	+123.04	32.9	71.7
Differences between treated and controls	0	+33.4	+19.4	+9.8	+43.6	—	—	71.7

Although all the treatments showed slight increases over the controls, none of these differences reached the level required for significance.

*Beau Champ*

	O.	N.	P.	K.	NPK.	General mean.	S.E. of difference between two means.	Significant difference.
Differences in the growth increments between preliminary and treatment periods	+86.4	+94.0	+103.4	+120.2	+95.2	+99.84	46.7	101.7
Differences between treated and controls	0	+7.6	+17.0	+33.8	+8.8	—	—	101.7



The treatments produced no significant responses: the significant difference is high in this experiment, apparently because the plants were somewhat old ratoons of White Tanna which suffered rather severely from borer.

*Indian Planter, Phoenix*

	O.	N.	P.	K.	NPK.	General mean.	S.E. of difference between two means.	Significant difference.
Differences in the growth increments between the preliminary and treatment periods . . .	-39.0	+4.8	+18.2	+31.4	+33.2	+9.72	25.9	56.4
Differences between treated and controls	0	+43.8	+57.2	+70.4	+72.2	—	—	56.4

In this experiment the responses to phosphate and potash were significant, but the response to nitrogen was not significant. A significant response to NPK was also obtained, but there was no evidence of any interaction.

*Union Park, S.E.*

	O.	N.	P.	K.	NPK.	General mean.	S.E. of difference between two means.	Significant difference.
Differences in the growth increments between the preliminary and treatment periods . . .	+108.6	+188.2	+105.0	+159.6	+172.6	+146.8	27.0	58.9
Differences between treated and controls	0	+79.6	-3.6	+51.0	+64.0	—	—	58.9

The treatments N and NPK produced a significant response: it would appear that nitrogen was the only deficient element.

Further measurements of the stools in all these trials during a third month resulted in identical conclusions to those recorded above.

## DISCUSSION AND SUMMARY OF PART II

[For Discussion and Summary of Part I see p. 16]

The direct method of determining fertilizer deficiencies from growth responses of the crop actually growing in the areas concerned appears to be an accurate and reliable method. In a period of 2 months it is possible to obtain indications of nutrient requirements, the accuracy of which appears to be at least equal to that of large-scale field trials. If carried out at the beginning of the period of active growth, the results obtained may be utilized for correct fertilization of the standing crop.

The advantage claimed for injection methods (see Pt. I pp. 1-15), on the ground that some elements may become fixed in an unavailable form in

the soil, is of little consequence in the case of sugar-cane, since application to the soil is the only practicable means of supplying this crop with essential mineral substances. The growth rate method has the advantage of being within the capacity of practical agriculturists, thus making it possible for estates to use the method to determine the fertilizer requirements of their own fields. It has also the considerable advantage of giving absolute and not comparative results, since the increased growth rate when responses are obtained is the direct forerunner of increased yields.

#### ACKNOWLEDGEMENT

I am indebted to my assistant Mr. A. d'Emmerez de Charmoy for considerable help during the course of these investigations.

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# On Diurnal Variations in the Mineral Content of the Leaf of the Cotton Plant<sup>1</sup>

BY

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With two Figures in the Text

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## I. INTRODUCTION

CURTIS (1935) remarks: 'It has been tacitly assumed by many that during the day, when transpiration is taking place at high rates, salts are rapidly carried to the leaves in the transpiration stream, that in the leaves the inorganic ions are combined with organic substances, and the resulting compounds are retransported from the leaves to regions of utilization or storage. A diurnal fluctuation in nitrogen and ash contents of leaves showing high contents during the day and diminished amounts at night would tend to support this assumption.'

It will be agreed, we think, that the only satisfactory basis for the expression of results is the absolute or sample basis, for when results are expressed as percentages, e.g. of fresh weight, dry weight, or residual dry weight, there is the possibility that the basis itself may show diurnal variation. Neither Gouwentak (1929), who used the half-leaf method, nor Denny (1932) nor Curtis (1935), who both used the twin-leaf method, found any significant evidence of diurnal variation in the nitrogen content of leaves. Curtis, however, quotes data obtained by Chang (1932), which indicate diurnal variations in the nitrogen content of bean leaves, but remarks that he is 'not convinced that they represent normal behaviour' owing to the conditions of the experiment.

<sup>1</sup> Paper No. 26 from the Physiological Department of the Cotton Research Station, Trinidad.

Mothes (1931) concluded that whether a diurnal effect occurs or not depends on the maturity of the leaf. With young leaves he found an import of nitrogen by day and by night, with mature leaves a gain by day and loss by night, and with senescent leaves a loss by day and by night. He used the half-leaf method, which is open to the objection that removal of half the leaf may influence the mineral supply to the remaining half of the leaf. Penston (1935, 1938) claimed to have demonstrated diurnal variation in the potassium content of potato and maize leaves. She used the sample basis, but no statistical data are given. The large and highly correlated changes in water, dry matter, and potassium suggest that imperfect sampling is the cause of the observed variation in potassium.

## II. PROCEDURE

Leaves judged to have just reached maturity were graded on the basis of node number and position in the field. The plants used were in a vegetative condition. Approximately 8,000 leaves were tagged. Collections were made at 6 a.m., noon, 6 p.m., and midnight on four consecutive days and a final collection at 6 a.m. on the 5th day. In order to ascertain the losses due to dew, samples were removed from the plant at 6 p.m. on the 2nd, 3rd, and 4th days of the experiment. The petioles were cut off and the laminae pinned to a branch in a position as near as possible to that occupied by the intact leaf. These leaves were collected at 6 a.m. on the following morning and will be referred to as the 'Off' group. Each collection contained three samples and each sample 100 leaves. No rain fell during the experiment, but there was heavy dew every night. Dew was removed with blotting-paper. Immediately after collections the petioles were cut off (where this had not already been done) and the samples weighed and then dried to constant weight in a steam-heated oven. Analyses were carried out on the dried material for total nitrogen, phosphorus, potassium, calcium, magnesium, and chlorine by methods previously described (Phillis and Mason, 1936).

## III. RESULTS

The results for fresh weight, dry weight, and water are shown in Fig. 1. Significant differences ( $P = 0.05$ ) are shown on the right of the figure. In each case the values are expressed as percentages of the mean value. It will be seen that the values all show an upward trend during the four days the experiment lasted. The diurnal changes in fresh weight were of the order of 5 per cent., a fact that would render fresh weight unsatisfactory as a basis for the expression of diurnal changes in the mineral content of the leaf. The changes in the moisture content are of course larger than the changes in fresh weight and tend to be balanced by changes in the dry weight. The leaf lost water only between 6 a.m. and midday, and gained water from midday to midnight. Between midnight and 6 a.m. there was either no change or a small uptake.



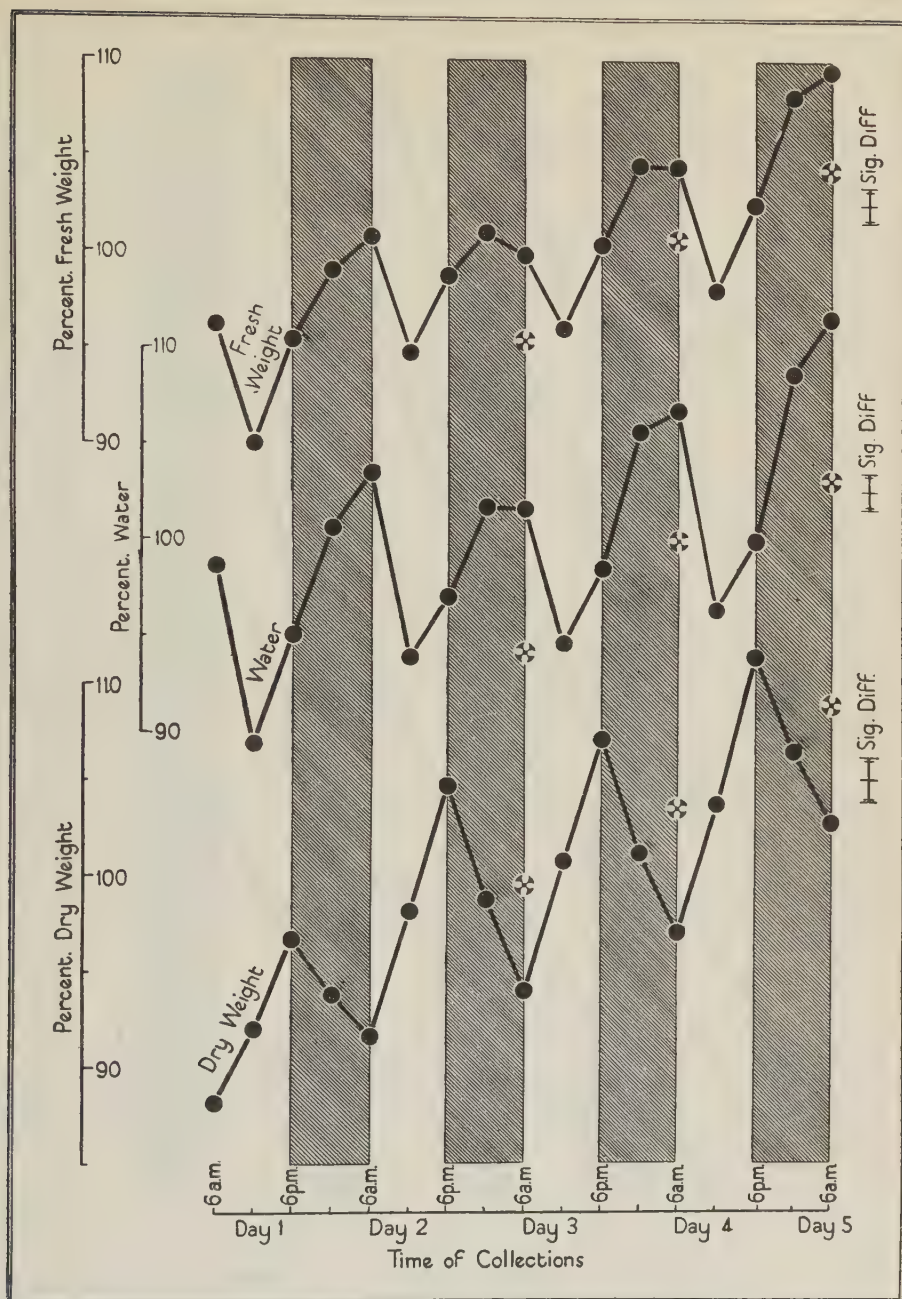


FIG. 1. Diurnal changes in fresh weight, water, and dry weight. Results for 'off' group are shown by crosses.

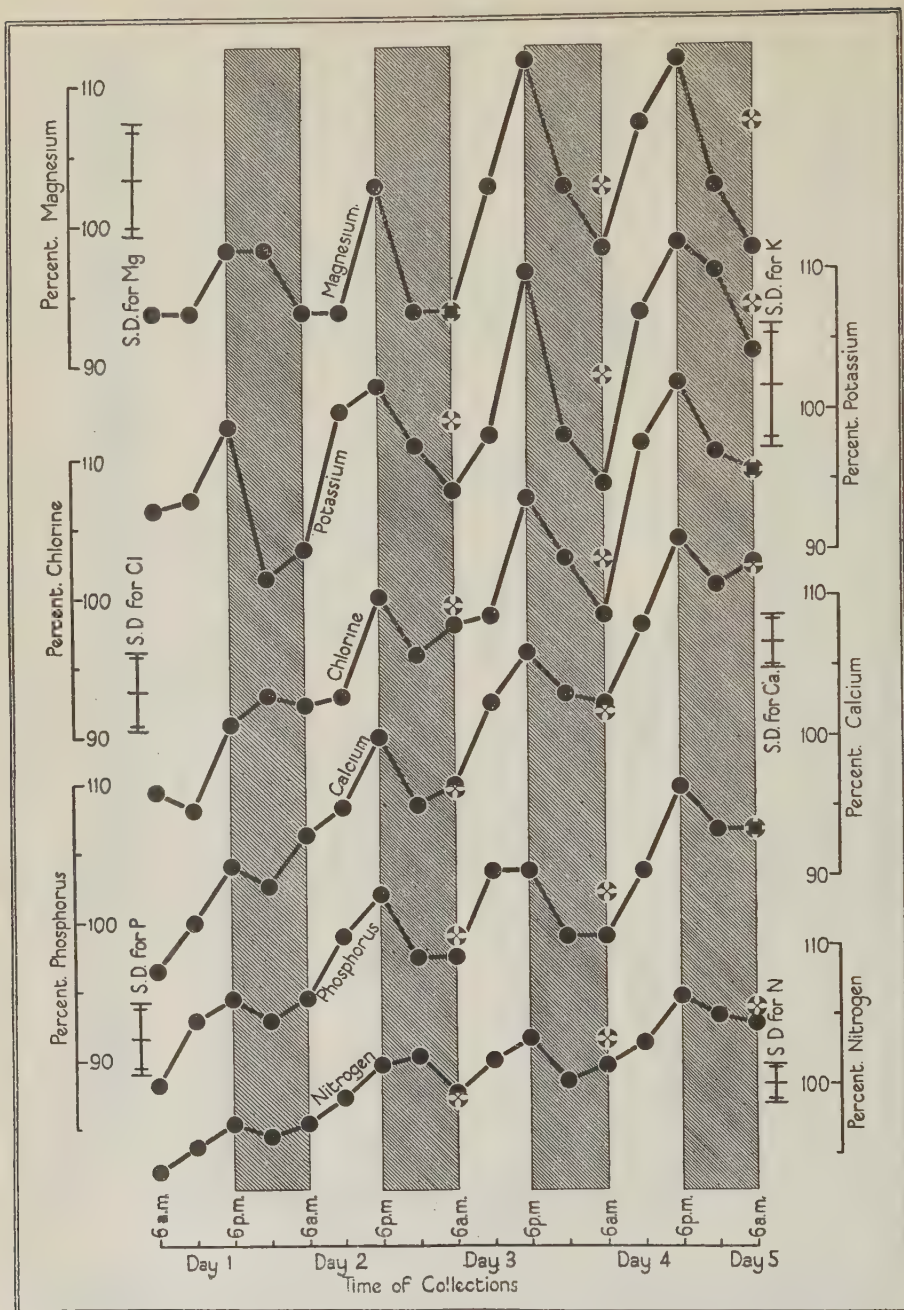


FIG. 2. Diurnal changes in the mineral elements. Results for 'off' group are shown by crosses.



The three 'Off' groups show, during the night, either no change or a small increase in water content. The dry weights of the 'normal' leaves, as might be expected, increased during the hours of daylight and diminished throughout the night. The dry weight of the 'Off' group showed about half of the loss of the 'normal' leaves, from which it would appear that the losses during the night resulting from export and from respiration were not greatly different.

The results for the mineral elements—nitrogen, phosphorus, potassium, magnesium, chlorine, and calcium—are shown in Fig. 2. The results are again expressed as percentages of the mean values. All these elements exhibit well-marked diurnal variations, increasing by day and showing either no change or a decrease by night. They also show an upward trend during the experiment. The variations were greatest for magnesium and potassium and least for nitrogen and phosphorus, while calcium and chlorine were intermediate.

We have now to consider the cause of these diurnal variations. (The results for the 'Off' group are shown by crosses in Fig. 2.) It will be seen that the values for the 'Off' group tend to be above those for the 'Normal' group except for calcium, where they tend to be slightly below the 'Normal' group. It is uncertain, however, how accurately the 'Off' group dew losses represent those in the 'Normal' group. The behaviour of calcium encourages, at first sight, the belief that the losses were the same in the two groups, but it must be remembered that the 'Normal' group was probably receiving some water from the transpiration current throughout the night and this transpiration water would undoubtedly contain calcium. This being so, it would appear that the 'Normal' leaves lost more calcium through leaching than the 'Off' leaves. The difference in the Normal/Off relationship between calcium (phloem immobile) and the other elements (phloem mobile) does suggest that phloem export through the petiole was responsible for some of the losses registered for the phloem mobile elements. There is, of course, the probability (cf. 'Off' group, Fig. 1) that the 'Normal' leaves absorbed some water from the dew on their surfaces.

#### IV. DISCUSSION

The results conform to the view that the mineral elements enter the leaf in the wood and with the exception of calcium are exported from it in the phloem. It is, of course, impossible to say whether the diurnal variations are limited to the veins or whether they also occur in the chlorenchyma. The losses indicated as a result of the dew are somewhat startling. Collections of dew from normal leaves were made about midnight and were found to contain an abundance of potassium with traces of calcium (cf. the losses of potassium and calcium indicated in Fig. 2). The pH of the dew was found to be about 8.5, which may help to explain why the potassium losses tended to exceed those of calcium. Large losses of potassium and smaller losses of calcium have been noticed by both Arens (1934) and Guyon (1937). The dew collected would appear to consist mainly of a weak solution of potassium bicarbonate. In making

collections of dew we made no attempt to limit the collection to the sampled leaves.

#### V. SUMMARY

1. Samples of leaves were collected at 6-hour intervals over a period of 96 hours, and their dry weights, water, and mineral contents determined, results being expressed on the sample basis.
2. There were well-marked diurnal changes in all these values.
3. The results are in harmony with the view that the mineral elements enter the leaf in the wood and, with the exception of calcium, are exported from it in the phloem.
4. The interpretation of the results is complicated by losses of mineral elements caused by dew.

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# Studies on Foliar Hydration in the Cotton Plant

## I. The Effects of Potassium Supply and Size of Plant<sup>1</sup>

BY

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With five Figures in the Text

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## I. INTRODUCTION

THERE is a considerable volume of data scattered through the literature on the relation between potassium supply and hydration. The most puzzling feature of this data is that different investigators have obtained diametrically opposite results. Thus, Gregory and Richards (1929) found that deficiency of potassium supply may lead to greatly increased succulence of foliage, while Janssen and Bartholomew (1929, 1930) showed 'That high potassium plants are more succulent than low potassium plants'. The literature on this subject has recently been critically reviewed by Richards and Shih (1940 and 1940 *a*), who conclude that the general constitution of the nutrient solution is of great importance. They subjected the data derived from an experiment on barley grown under twenty-two different combinations of nutrients to statistical analysis and conclude that the observed effects on

<sup>1</sup> Paper No. 27 from the Physiological Department of the Cotton Research Station, Trinidad.  
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succulence could be predominantly ascribed to sodium and to phosphorus and that potassium showed no appreciable relation to succulence.

One of the most obvious results of increasing the supply of potassium to plants starved of this element is an increase in size. *A priori* considerations suggest that under conditions of high aridity size might become a controlling factor in hydration and might entirely mask any direct effects of mineral composition. In the present paper we have re-examined, with this possibility in mind, a number of experiments in which the potassium supply was varied over a wide range. In the experiments presented foliar hydration is considered in terms of dry weight, but in one experiment the changes in water are also considered in relation to changes in dry weight and in protein nitrogen. Dry weight of whole plant has been used as a measure of size of plant. Fresh weight would have been preferable, but fresh weight values are not available for the roots.

## II. POT-CULTURE (*Experiment I*)

### A. Procedure.

Sea Island cotton plants were grown (under glass) in pots filled with sand. Each pot contained about 10 lb. of sand. Three or four seeds were sown in each pot, but the seedlings were thinned to one per pot a few days after germination. Culture solution was applied at the rate of 150 ml. per pot per week for the first month and after this similar amounts were given twice weekly. Once each week the sand was thoroughly leached with rain water. Water was added daily until the pots dripped. There were six levels of potassium supply and about fifty plants for each potassium level. The plants were approximately 9 weeks old when they were sampled. The compositions of the culture solutions used were as follows:

Level of potassium supply:	1.	2.	3.	4.	5.	6.	
Potassium	12.5	25	50	100	200	400	p.p.m.
Nitrogen	200	200	200	200	200	200	"
Phosphorus	60	60	60	60	60	60	"
Sodium	205	201	192	175	140	70	"
Magnesium	50	50	50	50	50	50	"
Calcium	120	120	120	120	120	120	"
Chlorine	294	295	298	307	323	354	"
Sulphur	145	147	149	155	156	189	"

Iron, 10 p.p.m.; boron, 2 p.p.m.; zinc, aluminium, and manganese, 1 p.p.m. each, were also added.

### B. Results.

In Fig. 1 are shown the results for dry weight of the whole plant (i.e. size) as well as water per 100 gm. *foliar* dry weight (i.e. hydration) and potassium per 100 gm. *foliar* dry weight. It will be observed that hydration and size are much more intimately associated than hydration and potassium. The correlation coefficient between hydration and size amounts to  $-0.977$  and is fully

significant, while that between hydration and potassium is only  $-0.647$ . We have examined the data of a number of similar experiments and this difference in the correlations has always been found to prevail.

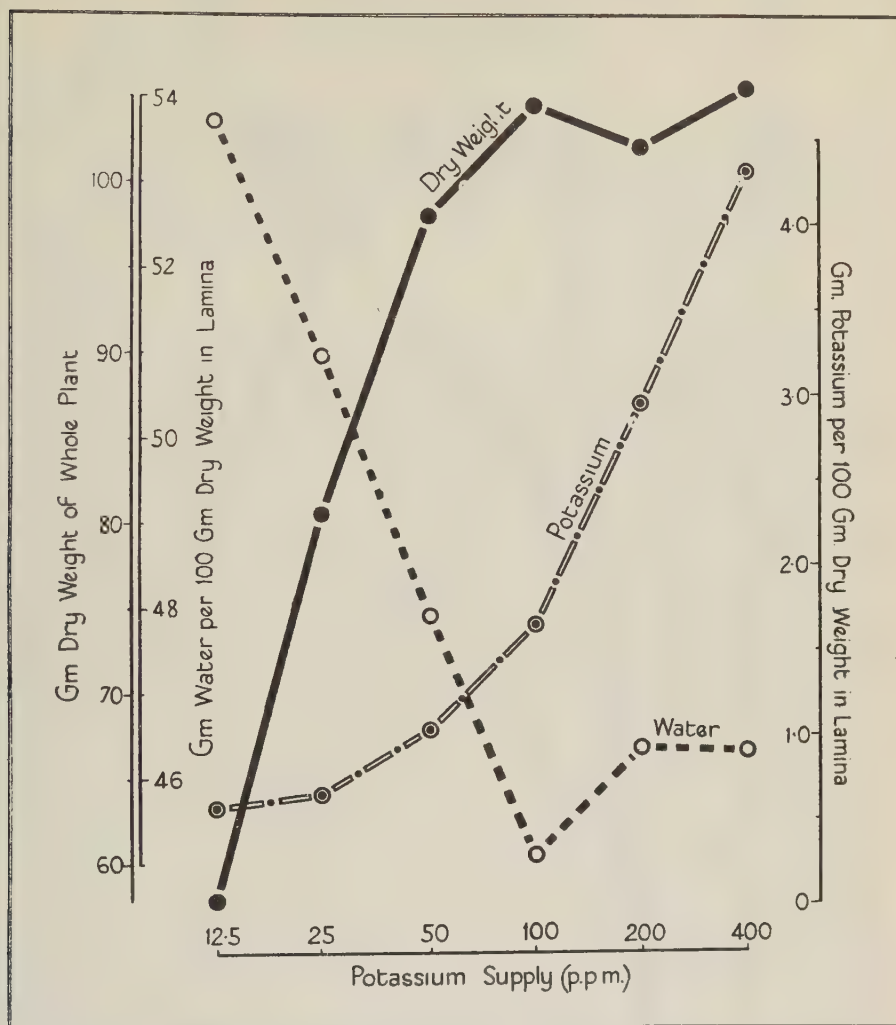


FIG. 1. Dry weights of whole plant per sample of 10 plants, and gm. water and potassium per 100 gm. dry weight in lamina, for varying levels of potassium supply to plants grown in pots (experiment 1).

The results for all such pot-culture experiments can be summed up as follows: As long as increasing potassium supply causes comparatively large increases in size of plant, foliar hydration decreases. This is what Gregory and Richards found. As the dry weight approaches a maximum value, however, this decline in hydration is stopped and then reversed. Further increases in

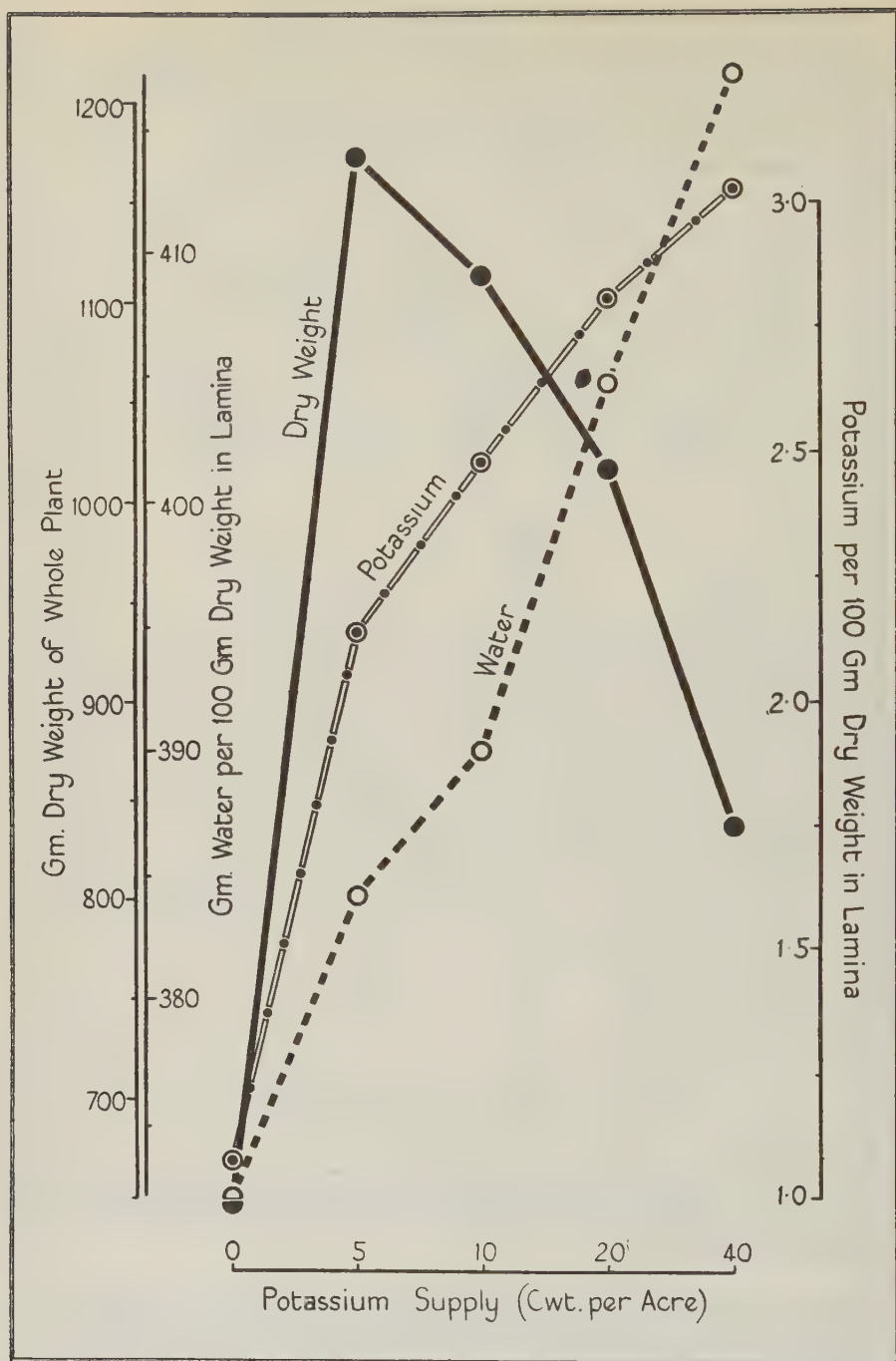


FIG. 2. Dry weights of whole plant per sample of 10 plants, and gm. of water and potassium per 100 gm. dry weight in lamina, for varying levels of potassium supply to plants grown in the field (experiment 2).



potassium supply above that required for maximum weight production cause increased hydration.

### III. FIELD-CULTURE (*Experiment 2*)

#### A. Procedure.

This experiment was carried out in the field. The treatments consisted of a control and four levels of potassium supply (5, 10, 20, and 40 cwt. of mixed potassium chloride and sulphate per acre). Each treatment was replicated five times. The plants were, as in the previous experiment, about 9 weeks old at the time of sampling.

#### B. Results.

The results are shown in Fig. 2, and again size, hydration, and potassium per 100 gm. dry weight are shown. It will be convenient to consider first of all the curves after the attainment of maximum dry weight, i.e. from the 5 to the 40 cwt. levels. Hydration is positively correlated with potassium and negatively correlated with size. The main interest of this experiment is, however, what occurred before the attainment of maximum size. It will be recollected that in the previous experiment for this part of the curve hydration was *negatively* correlated with both size and potassium. In this experiment it will be seen that hydration is positively correlated with both size and potassium.

To explain why hydration is at first reduced in pots and increased in the field with increasing potassium supply it may be suggested that in plants grown in pots the water strain is much greater than in those rooted in the soil. We have observed that plants grown in pots show a much greater tendency to wilt than plants rooted in soil in the field, although the aerial aridity is undoubtedly higher in the field than in the greenhouse. This difference in behaviour seems to be due to the subterranean humidity, which is probably much more variable and reaches much lower levels in pots filled with sand than in the soil in the field. Thus in pots an increase in size might cause big increases in subterranean aridity, whereas in the field it might have little or no effect. In pots root aridity appears to be the controlling factor in foliar hydration, completely overshadowing the potassium effect as long as size causes it to vary, whereas in the field root aridity appears to vary very little and so the potassium effect in increasing foliar hydration shows up.

### IV. FIRST PHOTOPERIOD EXPERIMENT (*Experiment 3*)

#### A. Procedure.

Sea Island cotton plants were grown in pots as in experiment 1. There were five levels of potassium supply and five different exposures to daylight. The potassium supply ranged from 12.5 to 200 p.p.m., and all plants received the

same volume of culture solution. The exposures ranged from full daylight (approximately 13 hours) through 11, 9, 7, and 5 hours per day. These exposures were spaced symetrically about midday. The plants were 8 weeks old when they were sampled.

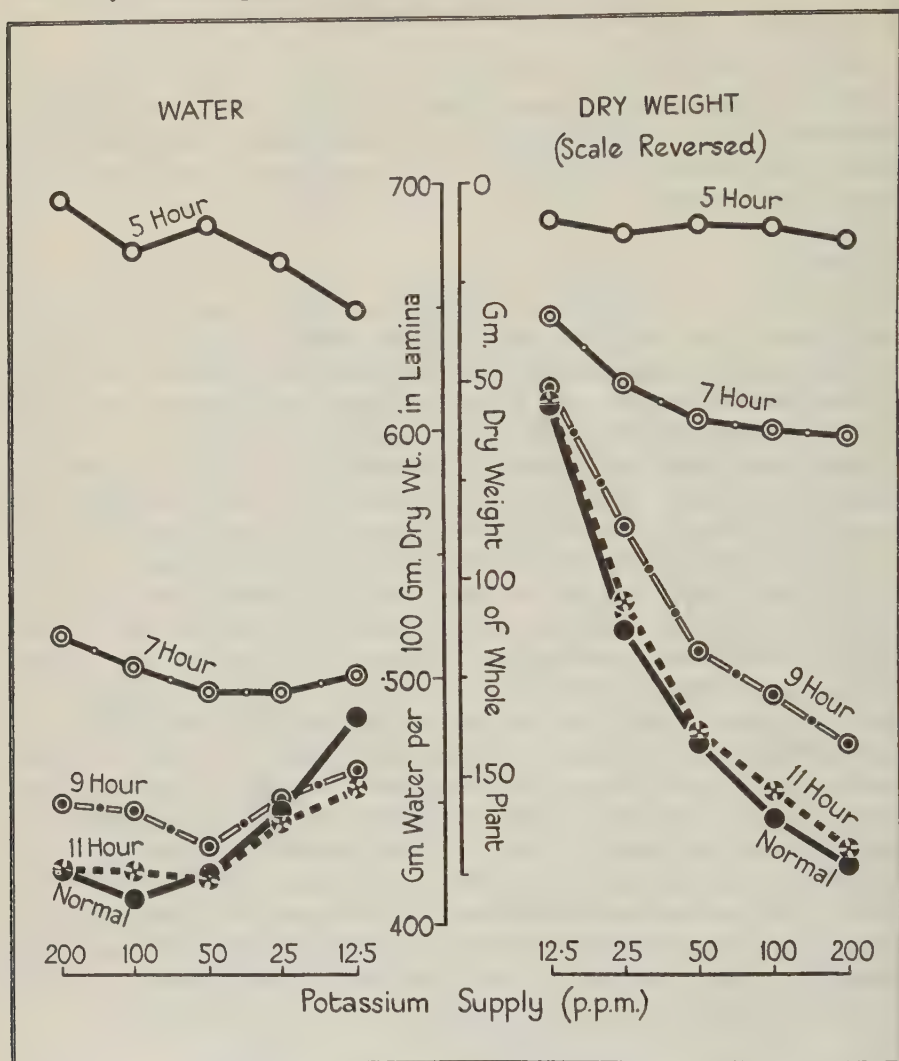


FIG. 3. Gm. water per 100 gm. dry weight in lamina (left), and gm. dry weight of whole plant per sample of 10 plants (right), for varying levels of potassium supply to plants grown in pots and given varying periods of exposure to daylight (experiment 3).

### B. Results.

The results for leaf hydration are shown on the left of Fig. 3, while those for size of plant are shown on the right; the scale for the latter is inverted. It will

be noted that the smaller the plants the greater generally is the hydration. The correlation coefficient between size and hydration for the whole series of data amounts to  $-0.96$  and is fully significant.

It will be seen that under short exposures the hydration tends to increase as the supply of potassium increases and that under long exposures the reverse obtains. It will also be observed that the response in size to potassium is much greater under long than under short exposures. The relative response is also rather greater under long than under short exposures.

The explanation of the reversal of hydration under short and long exposures may be as follows: The plants exposed to short periods of daylight show relatively small increases in size as the supply of potassium is augmented. The short day plants are also exposed for a shorter period to the desiccating influences of light, wind, &c. For both reasons the water strain need increase only slightly, if at all, as the potassium supply is increased. The potassium effect on hydration is therefore free to express itself and is not marked by any effects of changes in water strain due to changes in size. It will be seen that the potassium effect *per se* appears to be to increase the hydration as the supply is increased. The results for long exposures are similar to those already described in experiment 1.

## V. SECOND PHOTOPERIOD EXPERIMENT (*Experiment 4*)

### A. Procedure.

The procedure was generally similar to that of the preceding experiment, but there were only two periods of exposures, 6 hours and full daylight. There were again five different levels of potassium supply.

### B. Results.

The results are shown in Fig. 4. The plants exposed to 6 hours' daylight were not only smaller than those receiving full daylight, but the relative response to additions of potassium was also smaller. Increasing potassium supply led generally to an increase in hydration in the short day plants and to a decrease in hydration in the long day plants. The results are thus comparable with those of the preceding experiment. The correlation coefficient between hydration and size for the whole series of data amounts to  $-0.98$  and is fully significant.

If we interpret the results for hydration and size as in the preceding experiment it would appear that in the short day plants an increase in potassium supply causes an increase in hydration. In the long day plants, on the other hand, the considerable increases in size due to the addition of potassium brought about considerable increases in water strain which in turn led to decreases in hydration rather than to the increases which the changes in potassium *per se* might have been expected to produce.

We have hitherto considered hydration only in terms of dry weight. In

Fig. 5 we show the changes in the leaf in water, dry weight, and protein nitrogen. The results are expressed as percentages of the values at the lowest level of potassium supply. It will be seen that while there was no very marked

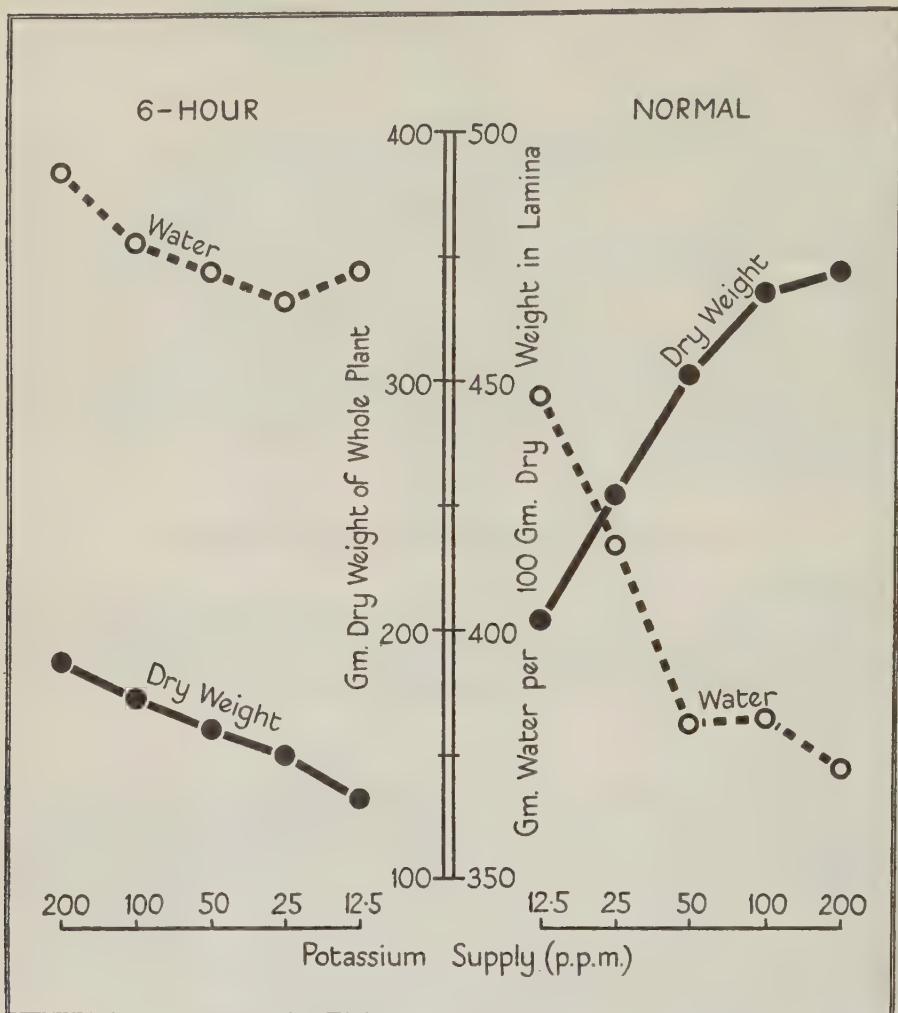


FIG. 4. Gm. water per 100 gm. dry weight in lamina and gm. dry weight of whole plant per sample of 10 plants for varying levels of potassium supply to plants grown in pots. Results are shown for normal daylight series (right) and 6-hour daylight series (left) (experiment 4).

difference in the behaviour of dry weight and of protein nitrogen in the leaves of the short and long day plants, water behaved very differently. In the long day plants increasing potassium supply was accompanied by a reduction in water, while in the short day plants there was an actual increase in water.



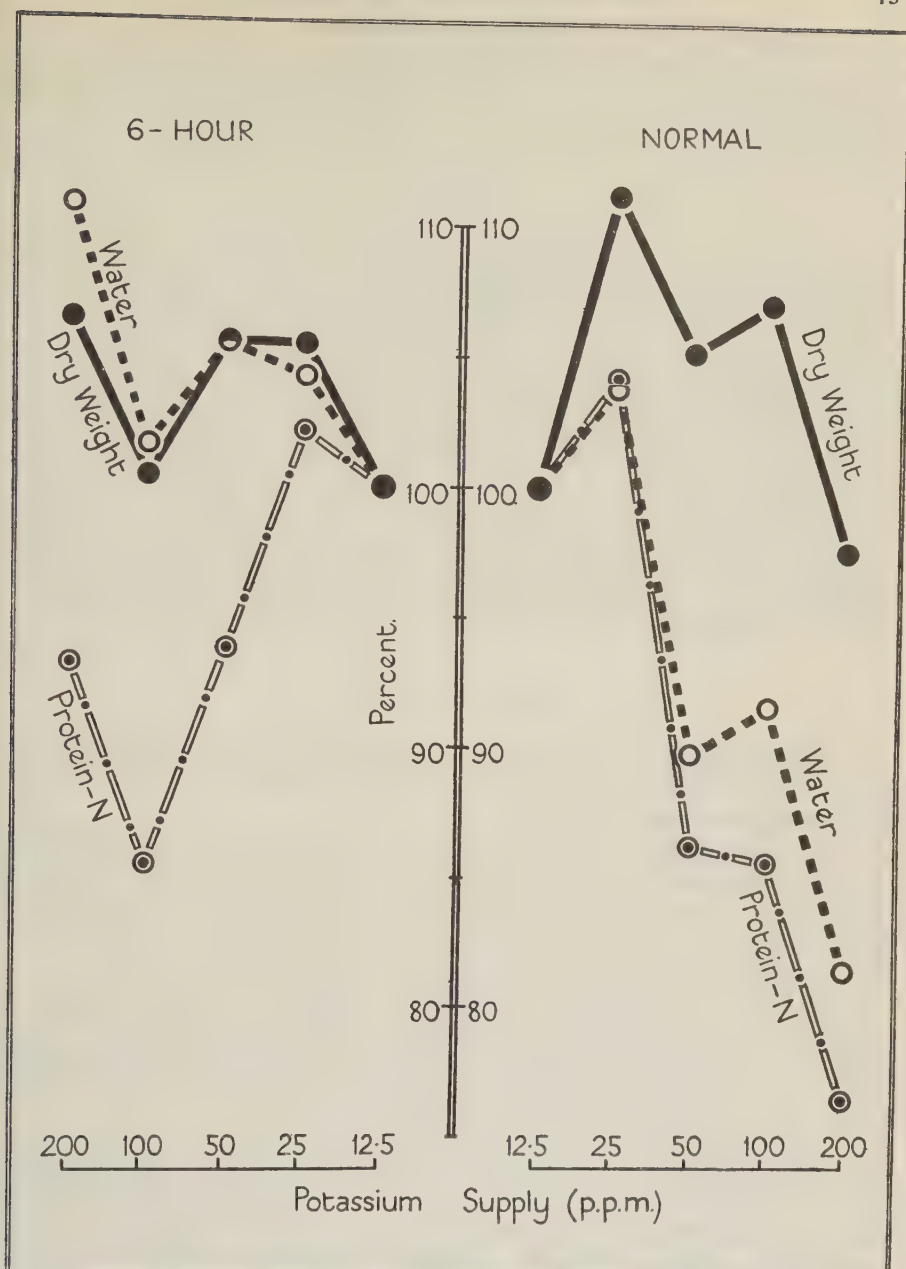


FIG. 5. Relative changes in lamina in water, dry weight and protein-N per sample of 10 plants for normal daylight series (right) and 6-hour daylight series (left) (experiment 4).

It is noteworthy that in both the photoperiod experiments increases in the potassium supply were accompanied by increases in the net assimilation rates in both the short and long day plants. Leaf hydration on the other hand was increased in the short and decreased in the long day plants.

## VI. DISCUSSION

The experiments reported in this paper suggest that differences of opinion that prevail concerning the relation between potassium supply and leaf hydration may in part be due to the failure to appreciate the importance of the size factor. It would appear that where increased potassium supply does not lead to marked increases in size the effect of potassium is to increase the hydration, but that where large increases occur it decreases hydration. Environmental aridity must be taken into consideration. Where humidity (soil or air) is high an increase in size is accompanied by an increase in hydration, but where humidity is low increased water strain resulting from increased size may outweigh the potassium effect on hydration. It is at present uncertain whether there is any specific potassium effect on hydration or whether other elements (sodium, cf. Richards and Shih, 1940) can also increase hydration.

## VII. SUMMARY

1. The published data on the relation between potassium supply and leaf hydration are contradictory, some investigators having found a positive correlation and others a negative one.
  2. It was found that an increase in potassium supply to plants growing in pots filled with sand led to a reduction in hydration and an increase in size of plant (size and hydration being negatively correlated), while with plants growing in the open in soil an increase in potassium supply led to increases in both hydration and size (size and hydration being positively correlated).
  3. To explain this difference in the effect of increased potassium supply on hydration in pots and in the field it is suggested that in pots an increase in size caused a reduction in hydration owing to the inadequate water-supplying power of the pots, while in the field an increase in size imposed no such stringent limitations on the water supply to the roots.
  4. To test this suggestion plants were grown in pots filled with sand under short daylight to ease the water strain, and it was found that under these conditions increased potassium supply was associated with increased size and increased hydration, while with controls growing under normal daylight increased size was associated with decreased hydration.
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# Studies on Foliar Hydration in the Cotton Plant

## II. Preliminary Observations using the Disc-Culture Method<sup>1</sup>

BY

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With five Figures in the Text

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### I. INTRODUCTION

‘EVER since the work of Pfeffer on osmotic pressure more than half a century ago, his view that the absorption of water is an osmotic phenomenon has dominated botanical thought on the question’ (cf. Stiles, 1936). The classical view of the mature leaf cell pictures it as contained within an elastic cell wall and consisting of a large central vacuole surrounded by a thin layer of protoplasm. The property of semi-permeability is attributed to the protoplasmic lining and by virtue of this, solutes contained in the vacuole are believed to exert an osmotic attraction for water.

The actual evidence for this view is meagre. It is based very largely on the relation between the concentration of the external solution causing incipient

<sup>1</sup> Paper No. 29 from the Physiological Department of the Cotton Research Station, Trinidad.

plasmolysis and that of the sap obtained after destruction of permeability. In general, fairly good agreement between these two values has been found (cf. Oppenheimer, 1932).

Bennet-Clark, Greenwood, and Barker (1936), however, have shown that in some cases there are big differences between these values and that in such cases the tissues are not plasmolysed by sap expressed from similar tissue and have postulated an active secretion of water from the external solution into the vacuole.

In all this work, the assumption was made that the sap represents the vacuole. Mason and Phillis (1939) have shown this assumption to be incorrect in the case of the cotton leaf and that the vacuole sap and the sap obtained on death of the protoplasm have widely different concentrations. In the cotton leaf the protoplasmic sap has a concentration from 5 to 10 times that of the vacuole, while the concentration of the vacuolar sap is only 15 to 20 per cent. of that of the whole cell. Thus, the difference between the solute concentration of the plasmolysing solution and that of the vacuole is much greater than was contemplated by Bennet-Clark, Greenwood, and Barker. These conclusions have recently been confirmed by Bennett-Clark and Bexon (1939).

Mason and Phillis have also shown that foliar tissues from which the vacuole has been expressed can absorb water and regain full turgidity. They have also demonstrated that in the leaf the volume of the protoplasm is about double that of the vacuole. These observations do not harmonize with the classical view of an osmotically active vacuole surrounded by a thin layer of protoplasm. Reinders' (1938) observation that oxygen promotes the uptake of water by the potato renders even more doubtful the view that the osmotic pressure of the vacuole is the sole force sending water into the cell.

In the present paper a new method of approaching the problem of hydration has been used. The method depends on a chance observation that discs punched from the cotton leaf and floated in daylight on salt solutions may increase their water content by *several hundred per cent.* This phenomenon is not confined to cotton leaves, but so far we have found no other plant which swells as readily as cotton. We have failed altogether to bring about swelling in monocotyledon leaves.

## II. METHODS

The experiments to be described were carried out with discs punched from cotton leaves. For punching,  $\frac{3}{4}$ -inch saddler's punches, carefully sharpened so that the discs were cut rather than bruised, were normally used. Care was taken to avoid the larger veins as well as areas of the leaf which were not flat. The discs were floated on salt solutions of various kinds and on water. The solutions and water were placed in large shallow trays (3 ft. square and 2 in. deep) which were filled almost to the brim and maintained at this level by the daily addition of water. The solutions were renewed at intervals of about

3 days. Each tray held approximately 1,200 discs. The experiments were carried out in daylight under glass. The daily range of temperature of the solutions was less than 5° C. Discs kept in darkness soon become injected and die. The discs were washed in water and dried with blotting-paper before weighing.

In all experiments, the fresh weights and dry weights of a known number of discs were determined. On sap expressed from frozen tissue, the specific conductivity at 0° C. was determined after diluting the sap 10 times. The conductivities recorded have been corrected for this dilution. The limitations of conductivity determinations on saps have been pointed out by Haynes (1919) and by Mason (1919). These limitations must be borne in mind in interpreting the results here presented. An *estimate* of salt content has been obtained from the water content and the conductivity. Freezing-point depressions were determined in most of the experiments. In one experiment the 'osmotic value' (cf. Bennet-Clark, Greenwood, and Barker, 1936) was determined on the lower epidermis.

### III. CHANGES IN DISCS FLOATING ON SOLUTION AND ON WATER OVER A PERIOD OF 24 HOURS (*Experiment 1*)

#### A. Procedure.

In this experiment the changes in water content and specific conductivity of sap from discs floating on water and on a full nutrient solution<sup>1</sup> were observed over a period of 24 hours.

#### *Sequence of events*

Day 0.	7.00 a.m.	Leaves collected and kept in dark with petioles in water.			
	7.15-8.45 a.m.	Discs punched and kept in covered tins.			
	8.45-9.00 a.m.	Initial collection of discs made and discs floated on solution and on water.			
	11.00 a.m.	Collection of discs from solution and from water.			
	1.00 p.m.	"	"	"	"
	3.00 p.m.	"	"	"	"
Day 1.	8.00 a.m.	"	"	"	"

#### B. Results.

The results are shown relatively in Fig. 1. It will be seen that after 23 hours the 'Solution' treatment shows a fully significant increase in water uptake over the 'Water' treatment. There was an initial rapid uptake of water by both treatments. After 3.00 p.m. the water uptake by the 'Solution' treatment forged ahead of the 'Water' treatment. It would appear that the initial rapid uptake by both treatments was due to the water deficit in the discs at the beginning of the experiment. As the external solution had a freezing-point

<sup>1</sup> The solution was similar to that usually employed by us for growing plants in water and sand cultures.

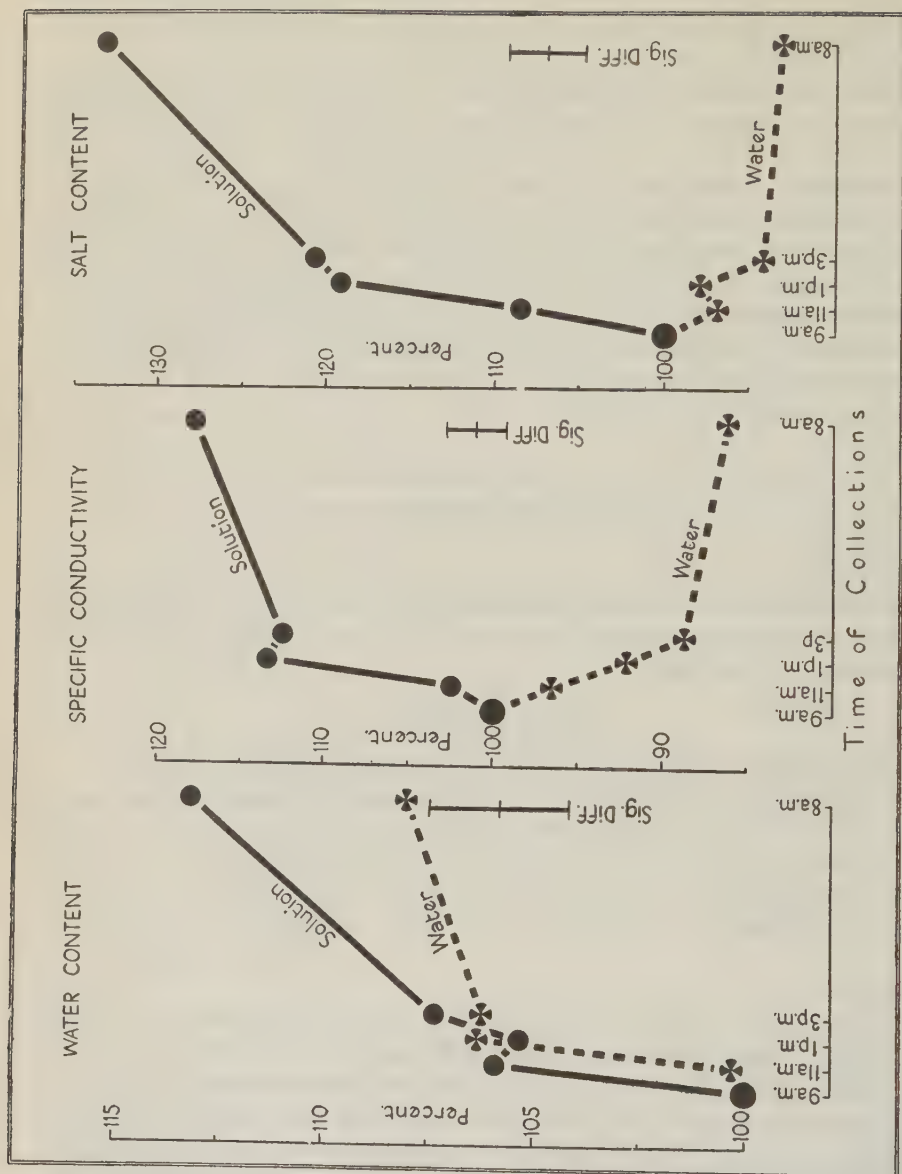


FIG. 1. Relative changes in water content, salt content, and specific conductivity of discs on water and on a nutrient solution.



depression of  $0.08^{\circ}\text{C}.$ , it is somewhat surprising to find that with the 'Solution' treatment water was taken up initially as rapidly as with the 'Water' treatment and that later it surpassed it.

The changes in conductivity, which are also shown in Fig. 1, make it clear that there were marked differences between the two treatments. There was an initial rapid increase in conductivity in the 'Solution' treatment and an initial rapid drop in conductivity in the 'Water' treatment. It would seem that these discs are able to accumulate salts. The actual conductivities of the external solution and of the saps are shown in Table 1. It will be noticed that the conductivities of the saps are much greater than that of the external solution

TABLE I

*Conductivities ( $C \times 10^3$ ) of External Solution and Saps Expressed from Discs of 'Solution' and 'Water' Treatments at Various Collections*

Sp. cond. of ext. sol.	Initial 9 a.m.	Specific conductivities of saps.			
		Coll. 1. 11 a.m.	Coll. 2. 1 p.m.	Coll. 3. 3 p.m.	Coll. 4. 8 a.m.
1.94	10.65	10.28	9.81	9.45	9.19
		10.91	12.06	11.99	12.54

and that the *increase* in the conductivity of the sap of the 'Solution' treatment at the end of the experiment was just about equal to the conductivity of the external solution. The difference between the rates of water uptake of the 'Solution' and 'Water' treatments does not appear until salts have been accumulated and the conductivity of the sap raised by an amount about equal to that of the external solution. Freezing-point depressions were unfortunately not carried out in this experiment.

The salt content of the discs is also shown in Fig. 1. It will be noticed that there was a marked loss of salts from the 'Water' treatment and of course a gain by the 'Solution' treatment.

#### IV. CHANGES IN DISCS FLOATING ON A SOLUTION FOR A PERIOD OF 13 DAYS (*Experiment 2*)

##### A. Procedure.

In this experiment the behaviour of discs growing on a nutrient solution similar to that used in experiment 1 was followed for a period of 13 days. In addition to changes in water content and conductivity of sap, the freezing-point depressions of the sap and the osmotic values (Og) of the lower epidermis were determined.

##### *Sequence of events*

Day 0. Discs punched, dropped into water, and dried after a few minutes. Discs for initial collection floated on water for 3 hours. Discs for later collections floated on solution.

Days 1, 2, 5, 8, 13. Collections of discs floated on Solution.

On the 8th day, discs for collection on the 13th day were cut into halves. The uptake of water by discs floating on solution slackens off after about a

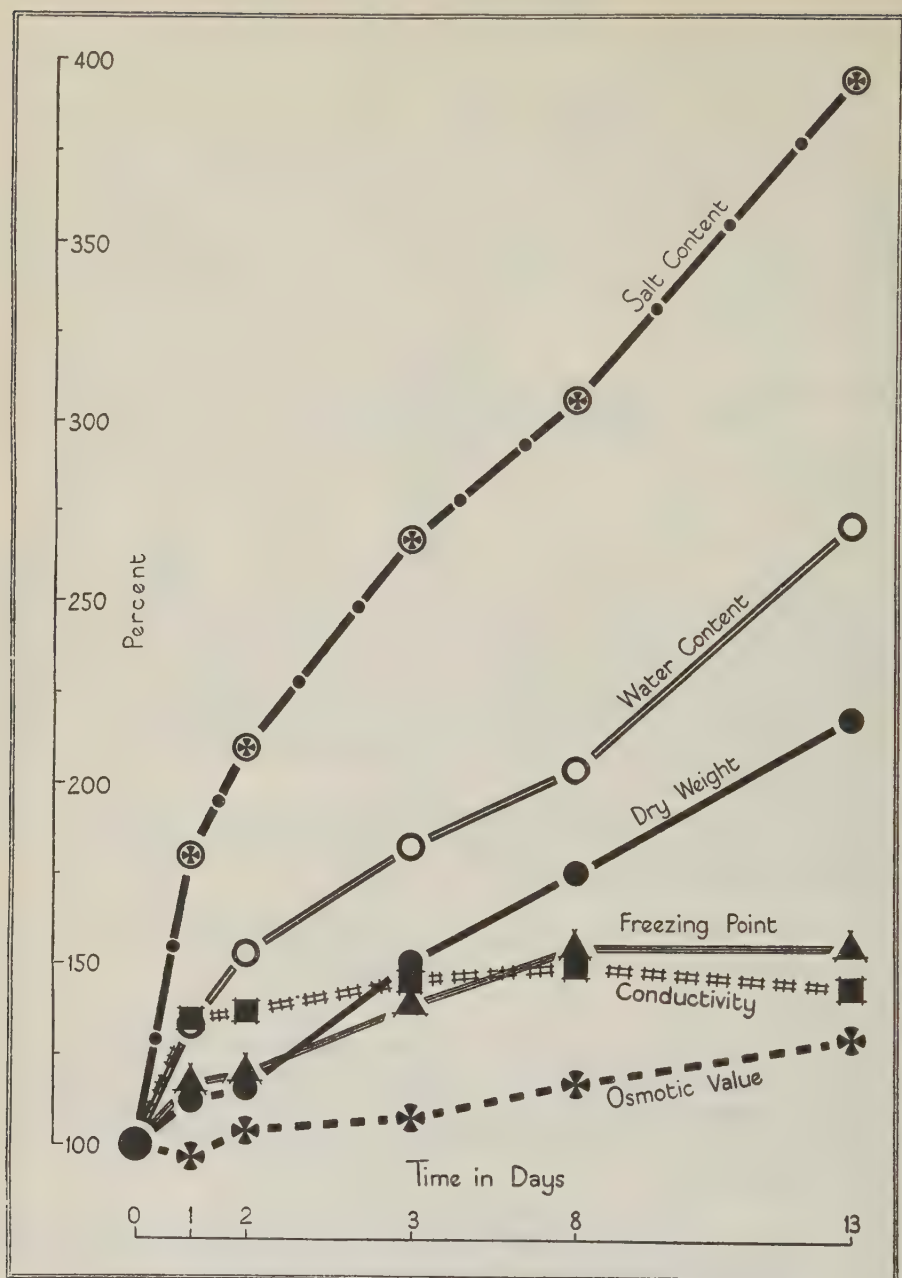


FIG. 2. Relative changes with time in the contents of water, dry weight, and salt, in the freezing-point depression and conductivity of the sap, and in the osmotic value of the lower epidermis of discs on a nutrient solution.

week apparently owing to callus formation. It has been found that rapid water uptake is resumed if the discs are cut.

### B. Results.

The results are shown in Fig. 2. They are as usual expressed as percentages of the initial values. The total increase in water content amounted to about 170 per cent. This very large increase in water was accompanied by smaller increases in dry weight, freezing-point depression, conductivity, osmotic value, and a larger increase in salt content.

Water uptake slackened off after the 1st day, and as a result of cutting the discs the rate again increased between the 8th and 13th days. Conductivity showed an initial rapid increase and then underwent a very gradual further increase up to the 8th day, followed by a small decline between the 8th and 13th days. It will be clear that the changes in water content and conductivity are quite different. The same is generally true of the changes in water content and freezing-point depression. The rapid uptake of water between the 8th and 13th days without any appreciable change in freezing-point depression is to be noticed. The results do not suggest that water uptake by the discs was due to an increase in the osmotic pressure of the sap.

The changes in water content were rather similar to those in salt content, but this of course is due to the fact that the salt content (water content  $\times$  conductivity) changes are due almost wholly to changes in water content, the changes in conductivity being relatively small. Protein changes were not determined, and there is the possibility that water uptake was due to an increase in the protein content of the cell.

The gross 'water-absorption' pressure (Og) (cf. Bennet-Clark, Greenwood, and Barker, 1936) increased by about 30 per cent. Elastic extension of the cell wall seems to have played an insignificant part in the increase in the volume of the cell, for the reduction in volume from turgor to incipient plasmolysis was of the same order in the initial and 13th day collections (about 5 per cent.).

To sum up, the nature of the internal changes that were responsible for the uptake of water are not clear. It would seem that changes in salt *concentration* (i.e. conductivity) in the sap cannot have been responsible. A change in the amount of protein (cf. Pearsall and Billimoria, 1939) or a change in the hydration of the protein (cf. Jordan and Shore, 1938) due to an increase in salts are among the possible causes. As we have not determined respiration, we are not in a position to discuss the possibility that salt may cause metabolic changes which are in some way responsible for the uptake of water (cf. Steward and Preston, 1940).

## V. THE EFFECT ON WATER ABSORPTION OF SALT CONCENTRATION AND TIME (Experiment 3)

### A. Procedure.

The procedure was essentially similar to that of experiment 2. There was, however, one important difference. Instead of a complete nutrient solution

(i.e. one containing all the essential elements), solutions containing only  $\text{CaCl}_2$  were used. There were four treatments. Discs were floated on water and on three  $\text{CaCl}_2$  solutions with freezing-point depressions of  $0.050^\circ \text{C}$ .,  $0.115^\circ \text{C}$ ., and  $0.215^\circ \text{C}$ . These solutions we will refer to as 1, 2, and 3 respectively. We have already remarked that water uptake falls off after some days and that it can be renewed by cutting the discs. The discs in this experiment were accordingly cut into halves at the time of collection 1, and into quarters at collection 2. There was an initial collection and further collections after 6, 10, and 15 days.

### B. Results.

The changes in water content, &c., are plotted against time in Figs. 3 and 4. In Fig. 3 each characteristic (water content, &c.) is considered separately, while in Fig. 4 the results for each treatment are considered separately.

It will be convenient to consider first the behaviour of the discs floating on the  $\text{CaCl}_2$  solutions and to postpone consideration of the water treatment. It will be observed that the uptake of water was greatest in the most concentrated solution and least in the most dilute. The maximum gain of water amounted to 169 per cent. The changes in dry weight were essentially similar for all treatments. The changes in dry weight are perhaps surprising as the various treatments had very different areas.

The changes in sap conductivity were in the main similar to those of water uptake but there were certain important differences. It will be seen that for the three solutions the rate of water uptake increased after the 6th day, while the rate of conductivity increase diminished after the 6th day. For solution 3 there was an actual drop in conductivity between the 10th and 15th days. The results do not suggest that water uptake is determined by the concentration of electrolytes. In particular, the changes in water and conductivity for solution 3 between the 10th and 15th days would seem to negative any theory of water uptake based on electrolyte concentration. It will be noticed that the changes in freezing-point depressions of the saps were very similar to the changes in conductivity and it would seem that the changes in freezing-point depression must have been mainly due to change in salt concentration. To sum up, it may, we think, be concluded that changes in osmotic pressure cannot have been the cause of water uptake by the discs.

Inspection of Fig. 4 shows that the changes in water content were *in pattern* most closely followed by the changes in salt content (calculated from water content and conductivity). The correlation coefficient between salt and water contents amounts to  $+0.99$ . Some degree of correlation between these two quantities might have been expected since water content is used in determining salt content, but as the changes in conductivity were, on the whole, much greater than those in water content, such a high correlation as  $+0.99$  could not have been anticipated. The closeness of the relation between salt and water contents is emphasized in Fig. 5, where the values for the three solutions and water are plotted on one graph. The points for the three collections for each



solution are joined together, and the regression line of water content on salt content, calculated for all the data, is also given. Inspection of the figure will

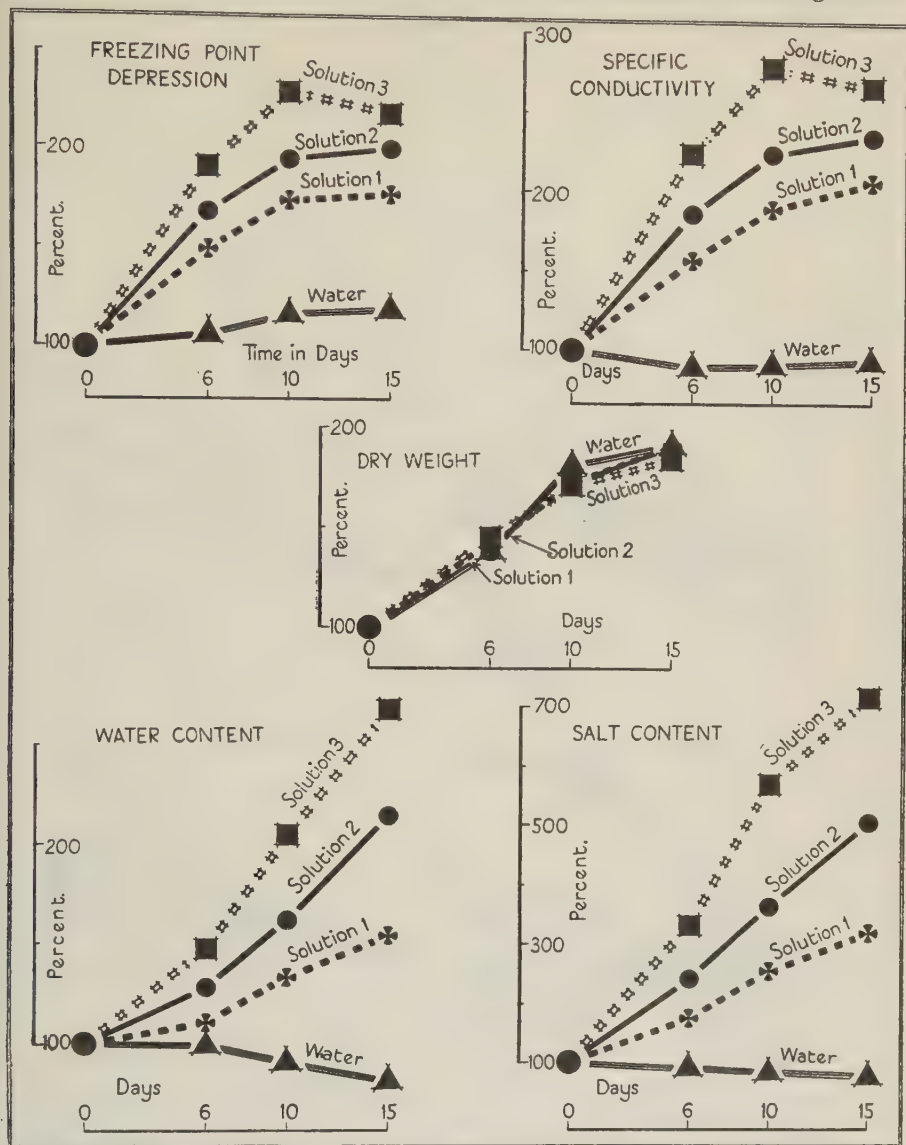


FIG. 3. Relative changes with time in the contents of water, dry weight, and salt, and in freezing-point depression and conductivity of discs on water and on three calcium chloride solutions. The changes are grouped according to the characteristic measured.

show that if three separate regression lines for collections 1, 2, and 3 had been drawn they would not have coincided, and that the line for collection 1 would have had the lowest slope and that for collection 3 the highest.

In this experiment there was no nitrogen in the solutions on which the discs were floating, so that differences in amounts of protein between the three salt

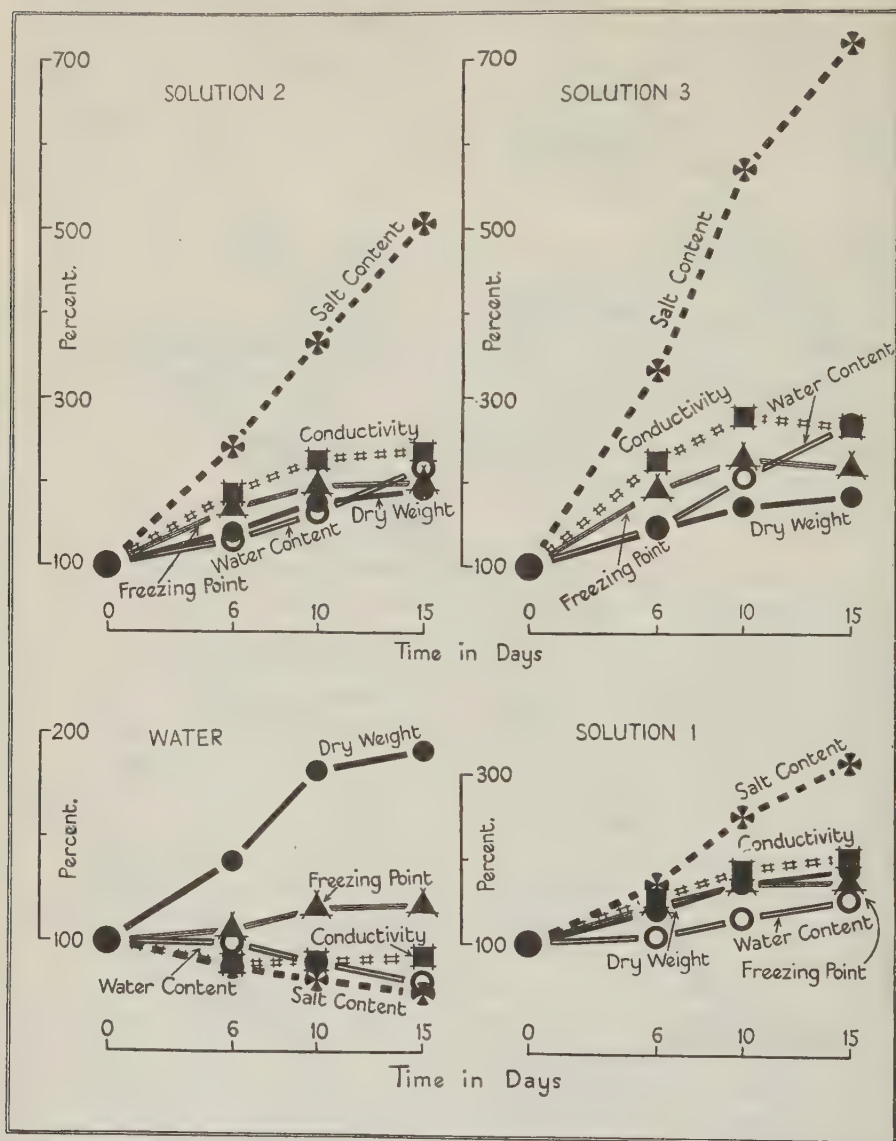


FIG. 4. Relative changes with time in water content, salt content, dry weight, freezing-point depression, and conductivity of discs on water and on three calcium chloride solutions. The changes are grouped according to treatment.

treatments can hardly have been responsible for the differences in the amounts of water taken up. The close correlation between salt and water contents could be accounted for if water uptake in the discs was controlled by the extent to

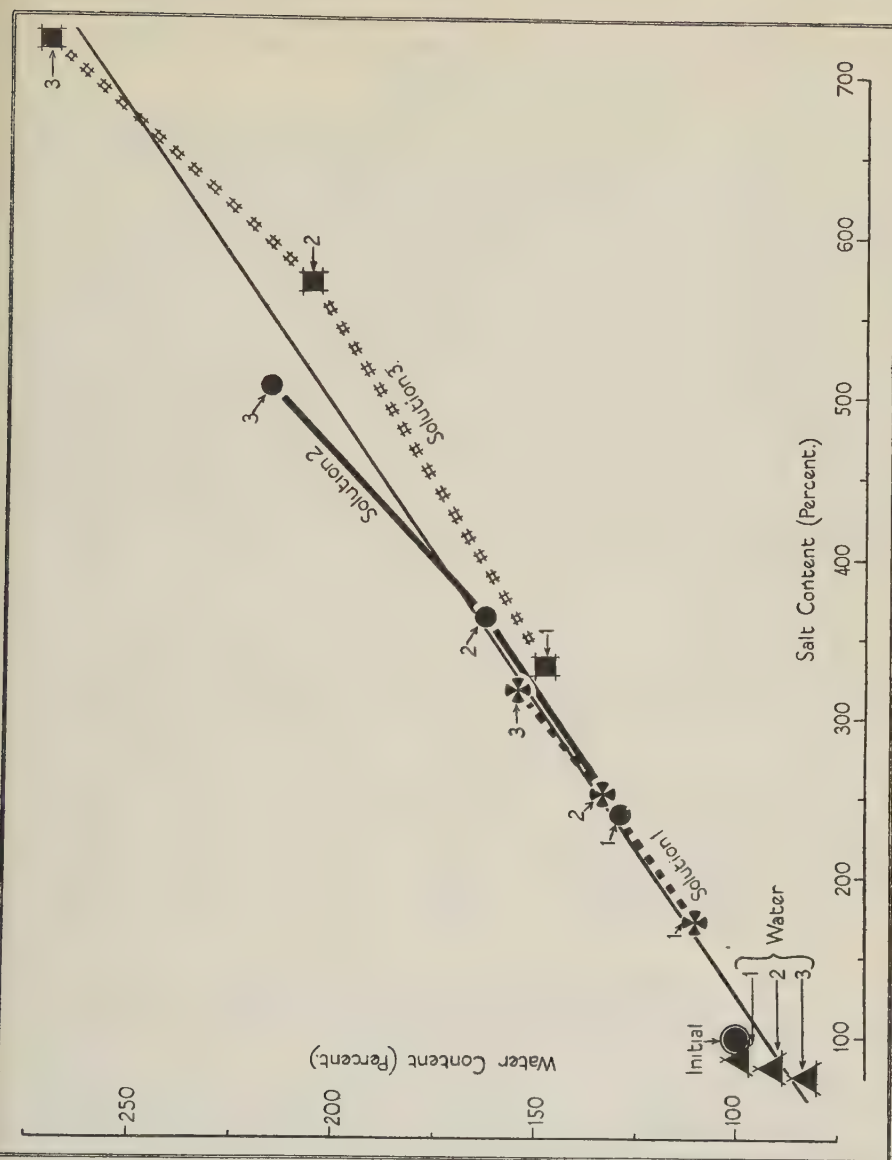


FIG. 5. Relative changes in water and salt contents plotted against each other. Numbers of collections are shown beside the points.

which the proteins were saturated with salt. On this view the changes in the slope of the regression lines between collections 1 and 3 would suggest that as the discs aged, their response to salt increased. Recent experiments, it may be added, support this suggestion of an age factor.

Before leaving the solution treatments, a word should be said concerning the actual concentrations in the external solutions on which the discs were floated and the *increase* in the expressed saps. The freezing-point depressions and the conductivities of the three external solutions and also the increases in freezing-point depression and conductivity of the sap between the initial and third collections are shown in Table 2. The increase in the sap of freezing-point depression and of conductivity are presumably due to the uptake of calcium chloride. It may consequently be concluded that the concentration of calcium chloride in the sap was many times that of the external solution. How this concentration was brought about is not clear. It may be that the solution is concentrated in the bundle-ends by transpiration and then diffuses into the mesophyll. On the other hand, there may have been active accumulation (cf. Steward, 1937; Hoagland and Broyer, 1936). In this connexion, it is of interest to find that the accumulation ratios (i.e. internal/external concentrations) diminished as the concentrations rose.

TABLE 2

*Freezing-point Depressions and Specific Conductivities ( $C \times 10^3$ ) of External Solutions and the Increases in these Values in the Sap between the Initial and 3rd Collections. Accumulation Ratios are shown in Italics*

	Freezing-point Depressions.			Specific Conductivities.		
	External solution.	Increase in sap.	Accumulation ratio.	External solution.	Increase in sap.	Accumulation ratio.
Solution 1	0.050° C.	0.473° C.	9.46	1.20	8.85	7.37
„ 2	0.115° C.	0.613° C.	5.33	2.34	10.47	4.47
„ 3	0.215° C.	0.723° C.	3.36	4.46	13.97	3.13

Finally we must consider the changes that occurred in the discs floated on water. The first point is that the increase in dry weight (see Fig. 3) was similar to that of the solution treatments. The changes in water content (see Fig. 4), on the other hand, were quite dissimilar, for the water treatments showed a loss of just 20 per cent. between the initial and final collections. The discs at the time of the final collection, however, were fully turgid. This is difficult to explain, for discs when allowed to dry out wilt when the moisture loss is much less than 20 per cent. The independence of moisture changes and freezing-point depressions is emphasized by the fact that the freezing-point depression increased while the water content was decreasing. It will be noticed that the conductivity showed an initial drop and then showed little change. Salt content showed a continuous decline. The increase in freezing-point depression was therefore presumably due to sugar concentration. If the loss of



water by the discs was due to leaching out of salts, then there seems to have been a lag between salt loss and water loss.

## VI. DISCUSSION

It will be evident that only very tentative conclusions can be drawn from this preliminary survey of the behaviour of leaf discs floated on salt solutions. The remarkable uptake of water would seem in some way to be conditioned by the amount rather than by the sap concentration of salts. Salts are known to cause an increase in swelling of water-imbibed gelatine in the iso-electric region, and it may be that we are dealing with a similar phenomenon in the leaf. There are, however, important differences between the swelling of the leaf and that of gelatine under the influence of salts. The swelling of the leaf for instance is much greater than that of gelatine. Then, the swelling effect of salts on gelatine is proportional to the logarithm of the external concentration, while for discs swelling<sup>1</sup> appears to be proportional to the amount of salt absorbed. There is also the possibility that salts in some way bring about metabolic changes which affect the hydration of the protoplasm. Further work is at present in progress on the relation between salt content and hydration in which attention is being paid to the amount of protein and to respiration.

The suggestion of a time factor raises a number of interesting questions. If salts increase protoplasmic hydration and if time emphasizes the effect, then we may have a clue to the cause of senescence in leaves, for we have noticed that the longevity of discs is generally proportional to the extent of swelling. It will be recollected that Molisch (1929) suggested that senescence of the leaf was largely due to the accumulation of salts. We are at present investigating this as well as a number of other points raised by this preliminary survey. For example, the nature of the changes that occur in the cell wall during swelling and shrinkage require clarification. Then there are a number of problems relating to the effect of pH, nature of salt, &c., on hydration; as yet there is no indication that non-electrolytes influence the uptake of water by discs.

## VII. SUMMARY

1. Discs punched from leaves were floated on water and salt solutions in daylight.

2. It was found that the discs floating on salt solutions may show very large increases in water content (about 170 per cent. in 13 days) and that discs floating on water may lose water (about 20 per cent. in 13 days). This water uptake by discs took place both on a full nutrient solution and on solutions of  $\text{CaCl}_2$ .

3. An estimate of the electrolyte concentration in the sap was obtained from conductivity measurements, while an estimate of total solute concentrations was obtained from freezing-point determinations.

<sup>1</sup> The swelling of discs in concentrated salt solutions has not yet been investigated.

4. It was found that water uptake might take place without any change, and even with a decline, in conductivity and in freezing-point depression.
5. Salt content (conductivity  $\times$  water), on the other hand, showed a very close relationship with water uptake.
6. It is suggested that salt increases the hydration capacity of the leaf proteins in the same way that the hydration capacity of gelatine is increased by salts in its iso-electric region. It is also pointed out that salt might affect respiration and that this might in some way influence the hydration capacity of protoplasm.

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# Studies on the Partition of the Mineral Elements in the Cotton Plant

## III. Mainly Concerning Nitrogen<sup>1</sup>

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With six Figures in the Text

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## I. INTRODUCTION

IN a recent paper Phillis and Mason (1939) suggested that the partition of nitrogen in the leaf of the cotton plant is in a large measure conditioned by the requirement of the tissues for this element. They assumed that the insoluble form (i.e. protein nitrogen) was more intimately concerned in growth than the soluble form (i.e. crystalloid nitrogen), so that under conditions of starvation a higher proportion was present in the insoluble form than was the case under more favourable nitrogen supply. They termed the percentage of the total amount present in the insoluble form the *partition index*. Over wide ranges of nitrogen, phosphorus, and potassium supplies to the roots they found the partition index in the leaf to be highly (negatively) correlated with nitrogen

<sup>1</sup> Paper No. 29 from the Physiological Department of the Cotton Research Station, Trinidad.  
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per 100 gm. dry weight. The correlation was, however, not linear. They concluded that the degree of saturation of the tissues with nitrogen was an important factor in determining its partition. Their conclusions concerning phosphorus were generally similar to those for nitrogen.

While their paper was in the press a review of protein metabolism in the plant by Chibnall (1939) appeared. He concluded his review with a statement that 'the overriding fact remains that we do not yet understand the reason why, when certain leaves are detached from the plant, protein decomposition can be detected within a few hours. If this is indeed due to interference with the mechanism of protein synthesis then one is tempted to suggest that some influence of the root system, possibly hormonal, is responsible for the regulation of the protein level in leaves.' Chibnall's suggestion that isolated leaves cannot synthesize protein is supported to some extent by the recent work of Pear-sall and Billimoria (1939), who found that while isolated portions of meristematic and extending tissue could readily form protein, isolated mature green leaves could not do so. They, however, admit the possibility of mature leaves producing protein when attached to the plant. Walkley (1940) has very recently shown that mature barley leaves attached to the plant can synthesize protein provided that the younger leaves are first removed. This might be regarded as further evidence for the hormone theory, in that protein synthesis in older leaves only occurs when younger leaves, which may act as sinks for mineral supplies and hormones sent up from the root, are removed.

Other views on protein regulation have been put forward by Paech (1935), Gregory and Sen (1937), and Richards (1938). Paech's view is that protein regulation is controlled by mass action, the reactants being active nitrogen (ammonia) and carbohydrate. Chibnall criticized this mass action view on the grounds that carbohydrate did not appear to play the dominant role in the equilibrium required by the theory. Gregory and Sen in discussing the relation between protein synthesis and degradation in the green leaf remark that 'Two alternative views are here possible, namely, (1) that protein synthesis is a reversal of proteolysis, the direction of the reaction being determined by mass action and the velocities of the reactions concerned; (2) that the two processes are quite distinct and are catalysed by separate systems of enzymes, the intermediate amino acids being possibly chemically different'. They rejected the first possibility as the relative concentrations of protein and amino acids do not tend towards constant values. In favouring the second view, they suggest 'that the carbon dioxide production is related to the rate at which a cycle of protein synthesis and proteolysis proceeds, the intermediate products of glycolysis being drawn into this metabolic cycle, and glycolysis is "regulated" by the utilisation of carbohydrate in this way'. Richards found a marked correlation between respiration rate and protein content and to explain this he suggested 'a reciprocal relationship between respiration and protein synthesis . . . so that a given rate of carbon dioxide evolution can maintain only a definite quantity of protein'. This view is reminiscent of the suggestion of



Mason and Phillis (1936) that the maintenance of protoplasmic structure requires a continuous supply of metabolic energy. Richards says 'It is true of course that other things being equal the rate of carbon dioxide evolution will depend simply on the amount of material respiring, and protein may be taken as a rough measure of this. But in the experiment under discussion, . . . the other things are very far indeed from equality, and yet the same simple quantitative relation between carbon dioxide and protein nitrogen holds throughout.' Because of this, he stressed the direct relation between respiration and protein metabolism rather than the indirect one, protein-respiring material-respiration. One objection to Richards's theory is that protein maintenance is not dependent on respiration, for protein is still maintained in dead (e.g. dried) tissue.

It will be seen that there are really two distinct issues. The first is whether the protein-crystalloid levels in the leaf are controlled by internal leaf factors or by external control of the type suggested by Chibnall. If the former is the case, what are the internal regulating factors? It is proposed to deal with both these points in this paper. Some consideration is also given to the regulation between soluble and insoluble phosphorus and soluble and insoluble carbohydrate.

## II. PROTEIN SYNTHESIS IN ISOLATED LEAVES (*Experiment I, Jan. 1941*)

In this experiment, discs punched from leaves of nitrogen-starved plants were fed with abundant nitrate (cf. Mason and Phillis, in press). The rate of protein synthesis in the discs was compared with that in leaves attached to the plant. The attached leaves were fed through the roots in the normal manner.

### A. Procedure.

Plants were grown in sand culture with a low nitrogen supply (30 p.p.m.). When they were 9 weeks old and had 15-16 internodes they were marked with wool on the middle internode. The plants were then pruned so that there 4 leaves were left immediately below the wool and 6 immediately above it. The main stem above the leaves and all fruiting branches were removed. The 4 lower leaves will be referred to as 'Bottom' leaves and the 6 upper ones as 'Top' leaves.

On day 0 the 'Top' and 'Bottom' leaves from 50 plants were collected separately. Discs of  $\frac{3}{4}$ -inch diameter were then punched from each set. The discs from each region were then divided into three samples, one of which served as an Initial sample and the other two were floated with their ventral surfaces in contact with a full nutrient solution containing 150 p.p.m. nitrogen supplied as calcium nitrate. These discs were exposed to daylight and will be referred to as the 'Off' group. Leaves still attached to the plant constituted the 'On' group. Two samples of 'Top' and of 'Bottom' leaves from each of 8 plants were collected on day 0 for the Initial collection and the nitrogen supply to the roots of the remaining plants was increased to 600 p.p.m. The nitrogen

was supplied as calcium nitrate. Collections were made of both discs (Off) and attached leaves (On) on days 5 and 8. There were at each collection approximately 1,000 discs in the 'Off' group, while in the 'On' group two samples were taken at each collection. The leaves from 8 plants constituted a sample.

When punching the discs the main veins were carefully avoided, and in consequence the main veins of the 'On' leaves were removed immediately after collection and only the mesophyll used for analysis. After the fresh weight of the sample had been determined, it was subdivided for dry-weight determination and for freezing for sap expression. Total nitrogen was determined on the dry material and crystalloid nitrogen on the expressed sap. From these determinations, protein nitrogen was calculated.

### B. Results.

The Initial nitrogen contents per 100 gm. dry weight in the 'Top' and 'Bottom' leaves for both groups were as follows:

		Nitrogen (gm. per 100 gm. dry weight).	
		'Top' leaves.	'Bottom' leaves.
'On' group	.	2.45	2.44
'Off' group	.	2.42	2.43

The differences between 'Top' and 'Bottom' and 'On' and 'Off' were quite insignificant.

The weights of total, protein, and crystalloid nitrogen expressed as percentages of the amounts in the Initial samples are shown for the 'On' and 'Off' groups for both 'Top' (left) and 'Bottom' (right) leaves in Fig. 1. Table 1 shows the extent to which the nitrogen taken up during the experiment was converted into protein. It will be seen that for the 'Top' leaves the increase in total nitrogen was much the same in both groups and that the rate of conversion into protein was almost identical in the two cases. For the 'Bottom' leaves, the 'Off' group showed a greater uptake of nitrogen and a greater amount of protein formed than the 'On' group. The relative protein formation in terms of nitrogen accumulated was a little greater in the 'Off' than in the 'On' group, but the 'Bottom' leaves whether 'On' or 'Off' the plant did not seem quite as efficient as the 'Top' leaves in forming protein. *There can be no doubt, however, that in this experiment the leaves off the plant were quite as capable of forming protein as leaves on the plant.*

TABLE I  
*Relative Protein Formation in Discs ('Off') and in Leaves ('On')  
expressed as Percentages of Increases in Total Nitrogen*

		Day 5.	Day 8.
'Off' group	} Top	76	77
'On' group		73	79
'Off' group	} Bottom	72	73
'On' group		61	61

This experiment has been repeated in modified form several times and the *invariable* result has been to show that detached leaves can readily form protein

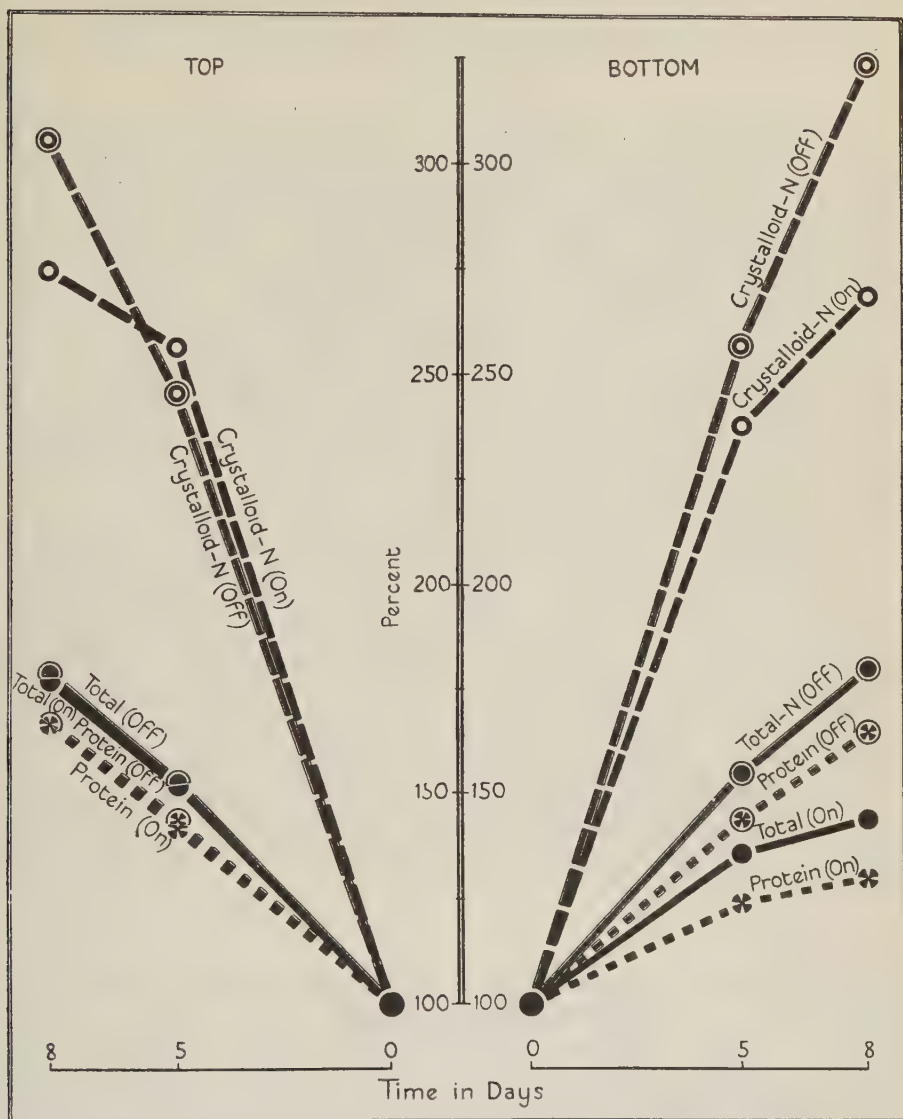


FIG. 1. Relative changes in the amounts of total N, protein-N, and crystalloid-N in 'On' and 'Off' groups for 'Top' (left) and 'Bottom' (right) leaves.

when supplied with inorganic nitrogen, and that the power of protein formation is not restricted in time to the period just after severance but persists undiminished for several days at least. It seems improbable, therefore, that

the root can play any part in regulation in the leaf through the agency of a hormone as Chibnall suggests.

### III. INTERNAL FACTORS REGULATING THE PROTEIN-CRYSTALLOID-N LEVEL IN THE LEAF

The experiment just described leads to the conclusion that the regulation of the protein-crystalloid-N level in the leaf must be due to factors in the leaf itself. Factors already suggested include the protein-crystalloid-N-sugar concentrations (Paech's mass action view) and respiration (Gregory and Sen, and Richards). There is no satisfactory evidence according to Chibnall that sugar plays any part in this regulation in the normal leaf, and Gregory and Sen (1937) ruled out the possibility of mass action regulation between crystalloid-N and protein-N because constant relative values could not be observed. On the other hand, the partition experiments of Phillis and Mason (1939) indicating that the degree of saturation of the tissues with nitrogen is an important factor in determining partition, suggest that the concentration of crystalloid-N may play a part and that there may be a saturation limit for protein, the relation between the two being governed by a law of diminishing returns.

In this section we have assembled data from four experiments covering a very wide range of conditions. These experiments include a sand-culture experiment with varying nitrogen supply, a water-culture experiment in which the nutrient concentration was varied, a sand-culture experiment in which the supplies of nitrogen, of phosphorus, and of potassium were each varied and in which leaf pruning treatments were applied, and a leaf-disc culture experiment in which discs punched from leaves were floated on a high nitrogen solution. These experiments were chosen because they each covered a considerable range of nitrogen, and together supplied an overlapping chain of data from very low to very high nitrogen levels. Experiment 1 is not included in this series because the range of nitrogen variation in terms of dry weight was small.

#### A. Procedure.

(a) *Experiment 2 (November 1938)*. Plants were grown in sand culture in two glass-houses at five levels of nitrogen supply (viz. 30, 60, 120, 240, and 480 p.p.m.). The two houses showed parallel but not identical results, and so the results are reported independently. The plants were kept in a vegetative state by the removal of flower-buds and were collected when they were 9 weeks old. The leaves were collected in three regions (Top, Middle, and Bottom), grading being carried out on node number. The results for the three regions are bulked in Figs. 2 and 3, but are treated independently in Figs. 4 and 5.

(b) *Experiment 3 (November 1940)*. Plants were grown in water culture in a greenhouse. The Normal culture solution contained 300 p.p.m. each of nitrogen and potassium, 100 p.p.m. each of calcium and magnesium, and



50 p.p.m. of phosphorus. In addition there were supplies of the trace elements and iron. There were two series of plants.

(1) Variable-Nitrogen series: The concentration of all nutrients (with the

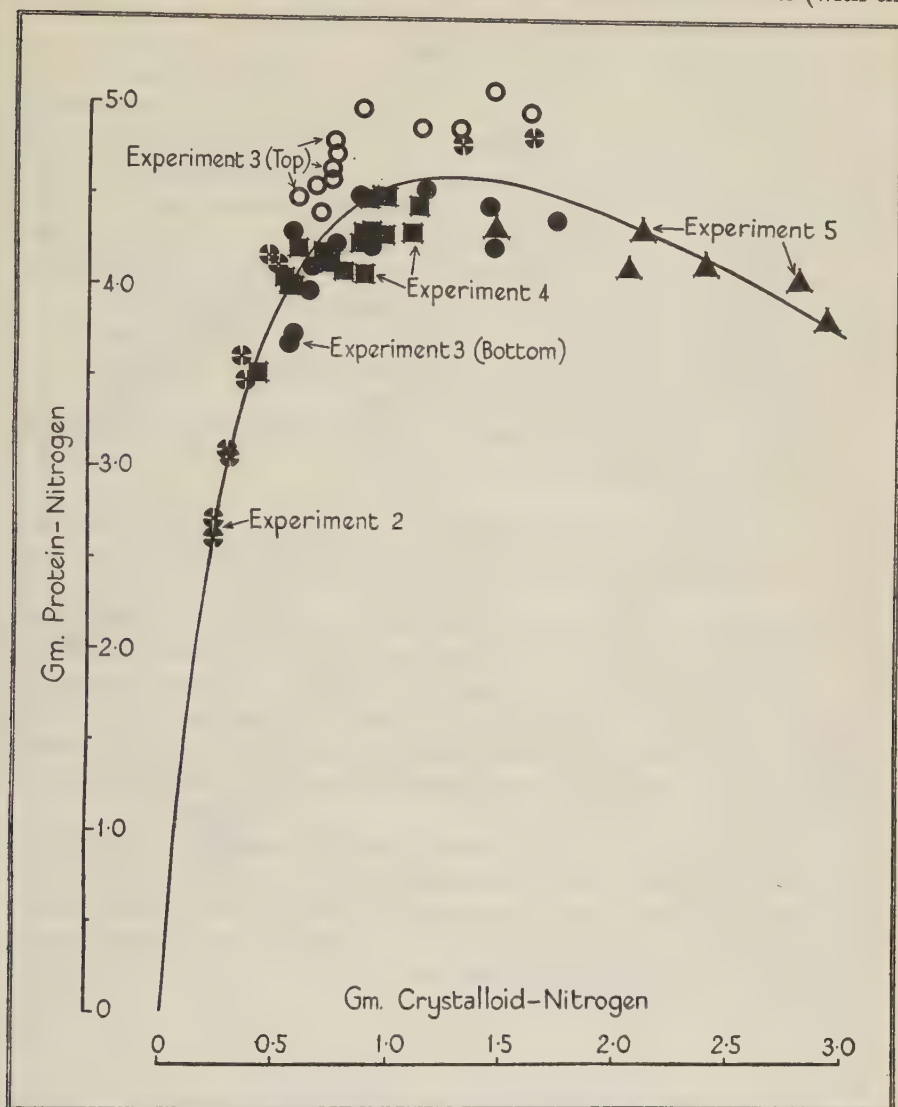


FIG. 2. Protein-N plotted against crystalloid-N per 100 gm. dry weight for experiments 2, 3 ('Top' and 'Bottom'), 4, and 5. The curve has been calculated from the regression line of partition index on Crystalloid-N per 100 gm. dry weight (see Fig. 3, right).

exception of iron and the trace elements) including nitrogen was varied from half to five times the Normal concentration. There were actually six levels of supply— $\frac{1}{2}$ N, 1N, 2N, 3N, 4N, and 5N.

(2) Constant-Nitrogen series: The concentration of all nutrients *except* nitrogen, iron, and the trace elements was varied from  $\frac{1}{2}$ N to 5N. Nitrogen was maintained at 300 p.p.m.

The plants were collected when they were 7 weeks old. Top and Bottom leaves were collected and analysed separately.

(c) *Experiment 4 (September 1941)*. Plants were grown in a glass-house in sand culture. There were six nutrient treatments, viz. high and low nitrogen, potassium, and phosphorus respectively. In the two nitrogen treatments the levels were 300 and 100 p.p.m. respectively (with potassium at 100 p.p.m. and phosphorus at 50 p.p.m.). In the two potassium treatments the levels were 160 and 80 p.p.m. respectively (with nitrogen at 200 p.p.m. and phosphorus at 50 p.p.m.), while in the two phosphorus treatments the levels were 320 and 20 p.p.m. (with nitrogen at 200 p.p.m. and potassium at 100 p.p.m.).

When the plants were 7 weeks old each of the six mineral treatments was subdivided into three groups which were treated as follows:

- (1) Concentration of nutrient supply doubled.
- (2) All leaves except those on nodes 5, 6, and 7 removed. (Approximately 80 per cent. of the leaves were removed.)
- (3) Control group.

Leaves on nodes 5, 6, and 7 in groups 1 and 3 were marked with wool. Eight days later these leaves were collected from each of the 18 groups of plants.

(d) *Experiment 5 (March 1941)*. Two series of plants were grown in sand culture at potassium supply levels of 50 and 300 p.p.m. respectively. When the plants were 9 weeks old, discs were punched from the leaves on nodes 5, 6, and 7 in each series. These discs were floated on a nutrient solution containing all nutrients except potassium, with nitrogen at 300 p.p.m. There was an initial sample of discs taken at the time of floating and three further collections were made at three-day intervals.

In all these experiments the leaf material after determination of fresh weight was subdivided for dry-weight determination and for freezing for sap expression. Crystalloid nitrogen was determined on the sap by the Kjeldahl method after clearing with colloidal ferric hydroxide. Total nitrogen was determined by the Kjeldahl method on dried material.

## B. Results.

The combined results for these four experiments are shown in Fig. 2, where protein-N is plotted against crystalloid-N, both expressed per 100 gm. dry weight. The first point of interest is the remarkable manner in which the points for any one experiment merge into those for other experiments, the whole set forming a single curve. The method of calculating the curve will be described later. It must be emphasized that these points cover a range from acute nitrogen starvation (expt. 2, low nitrogen levels) to considerable excess

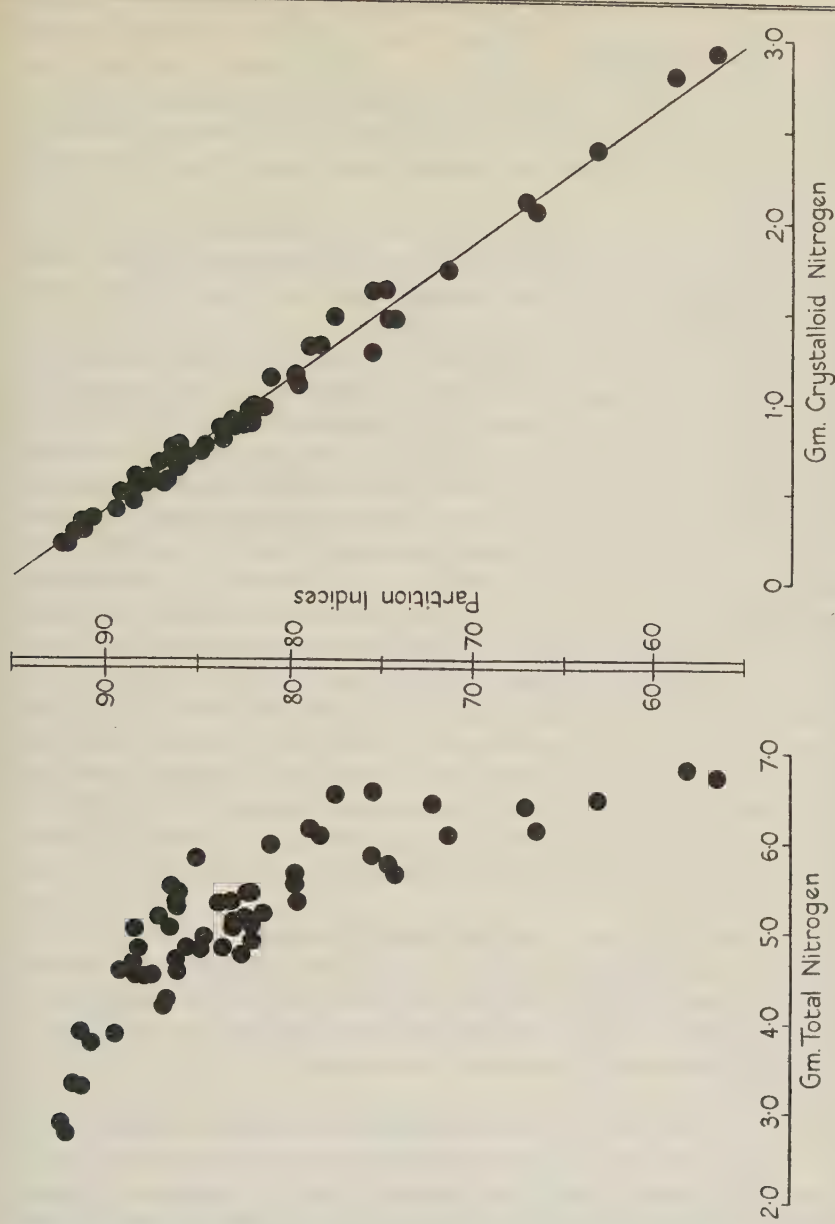


FIG. 3. The partition indices for nitrogen plotted against total-N (left) and crystalloid-N (right) per 100 gm. dry weight for experiments 2, 3, 4, and 5. The regression line of partition index on crystalloid-N is also shown.

(expt. 5). Actually, total nitrogen ranged from under 3.0 gm. per 100 gm. dry weight to 7.0 gm. per 100 gm. dry weight, a much greater range than that considered by Phillis and Mason (1939). The second point of interest is that protein-N first increases with increasing crystalloid-N, then reaches a maximum value, and beyond that declines. The view that the protein-crystalloid-N levels are connected by something akin to a law of diminishing returns seems to be untenable.

The remarkable manner in which the results for crystalloid-N and protein-N per 100 gm. dry weight for the four experiments form a single curve in spite of the variation in experimental conditions indicates that the controlling factor must be the crystalloid-N level.

Phillis and Mason (1939) correlated the partition indices (see introduction) with total nitrogen per 100 gm. dry weight. If crystalloid-N and not total-N is the controlling factor, correlation with crystalloid-N might be better. In Fig. 3 we show the relation between total-N per 100 gm. dry weight and the partition indices (left), and that between crystalloid-N per 100 gm. dry weight and the partition indices (right). As before, the relation between the partition indices and total-N is clearly not linear. Expressing total-N in terms of water or of protein-free dry weight (i.e. dry weight—5.7N) instead of in terms of dry weight does not affect the non-linearity. For crystalloid-N on the dry-weight basis, the relation with the partition indices is, so far as we can judge, linear, the correlation coefficient reaching the very high level of  $-0.994$ . When crystalloid-N is expressed in terms of water or protein-free dry weight the correlations are reduced to  $-0.860$  and  $-0.856$  respectively. The high correlation between partition index and crystalloid-N expressed on the dry-weight basis is positively startling when one considers the complexity of the nitrogen fractions and of dry matter, and also the great diversity of treatments, leading to wide variation in nitrogen content and partition index, over which this relation holds.

To sum up, it would appear that once the concentration of crystalloid-N in terms of dry weight is known, the partition index can be estimated with considerable exactness. The effect of great alterations in the mineral supply to the roots, of pruning, severance of leaf from rest of plant, &c., are all subordinate to the crystalloid-N concentration as factors determining the protein level in the leaf.

The linearity of the relation between the partition index and crystalloid-N has enabled us to calculate from the regression equation for these variables the protein levels for various crystalloid-N levels. The calculated curve relating protein-N and crystalloid-N (both expressed in terms of dry weight) was given in Fig. 2 along with the observed values. Considering the range the experiments cover, the agreement must be regarded as good.

Gregory and Sen dismissed the possibility that there might be a mass action basis for the regulation of protein-crystalloid-N levels on the grounds that they could obtain no evidence of a tendency towards a constant ratio. Such



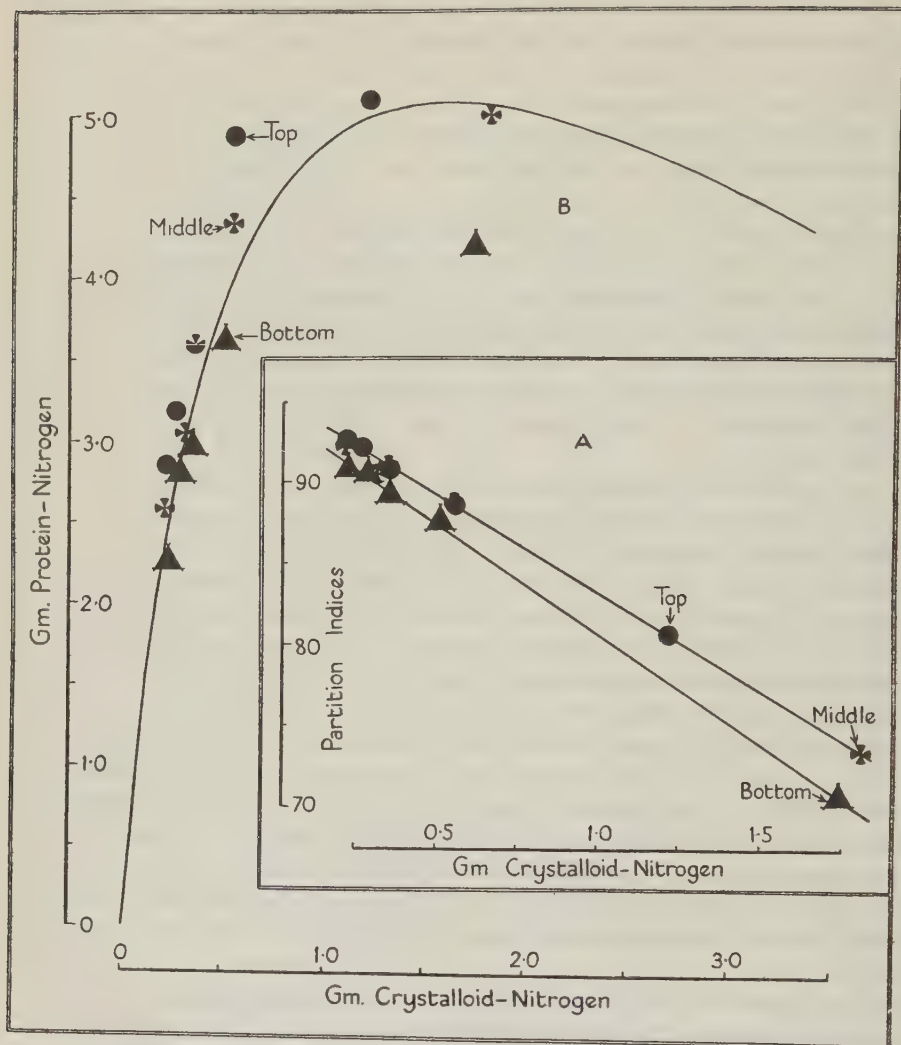
constancy would only be expected if the two reactants were in the same phase, which may not be the case for crystalloid-N and protein-N in the leaf. If crystalloid-N is adsorbed before conversion into protein, constancy of the protein-crystalloid-N ratio would not be expected. Buzagh (1937) lists several types of relation between concentration and adsorption, the most common type of apolar adsorption being one in which the amount of material adsorbed rises at first with increasing concentration, reaches a maximum, and then at still higher concentrations falls off; i.e. much the same behaviour as we have noted for the protein-crystalloid-N relation. According to Buzagh (p. 183) the most important signs for the recognition of apolar adsorption are: (1) the adsorption is generally reversible; (2) a condition of equilibrium is established in a short time; (3) if the results of measurements are graphically represented, the equilibrium concentration per unit volume of the adsorbed substance being plotted as abscissae and the quantity of substance adsorbed as ordinates, characteristic curves are obtained depending on the nature of the system. The most general form rises to a maximum, and decreases with further increase of the concentration. It may therefore be tentatively suggested that adsorption plays a dominant role in determining the equilibrium between protein and crystalloid-N.

We have already referred to Richards's conclusion that protein is maintained by respiration and think it more likely that the correlation ( $r = 0.711$ ) he found between respiration and protein was really due to the fact that in protein he had a measure of respiring material. We have taken all his data and calculated the correlation coefficient between the partition indices and crystalloid-N per 100 gm. dry weight. It amounts to  $-0.911$ . His data are therefore in harmony with our view that adsorption plays a dominant part in the regulation of protein-crystalloid-N levels.

In Fig. 2 the 'Top' and 'Bottom' leaves of Experiment 3 are shown separately and it may be seen that there is a small age effect. It will be noted that the 'Bottom' leaves are generally below the calculated curve, while the 'Top' leaves fall above it. In experiment 2 the leaves were collected in three different age groups, but in Figs. 2 and 3 a weighted mean value was used for each nitrogen supply level. The results for the three ages are shown individually in Fig. 4, where (A) the partition indices are plotted against the concentrations of crystalloid-N per 100 gm. dry weight, and (B) protein-N per 100 gm. dry weight is plotted against crystalloid-N per 100 gm. dry weight. In order to avoid confusion, we have shown the results for one glass-house only, the results for the other house being essentially similar. It will be seen that there is again a small effect of age which expresses itself in a greater ease in the young than in the old leaves in forming protein. It must be emphasized, however, that this age effect is very small when compared with the crystalloid-N level effect. It will be remembered that in experiment 1, where the protein-forming power of attached and detached leaves was compared, old leaves did not form protein quite as readily as young leaves.

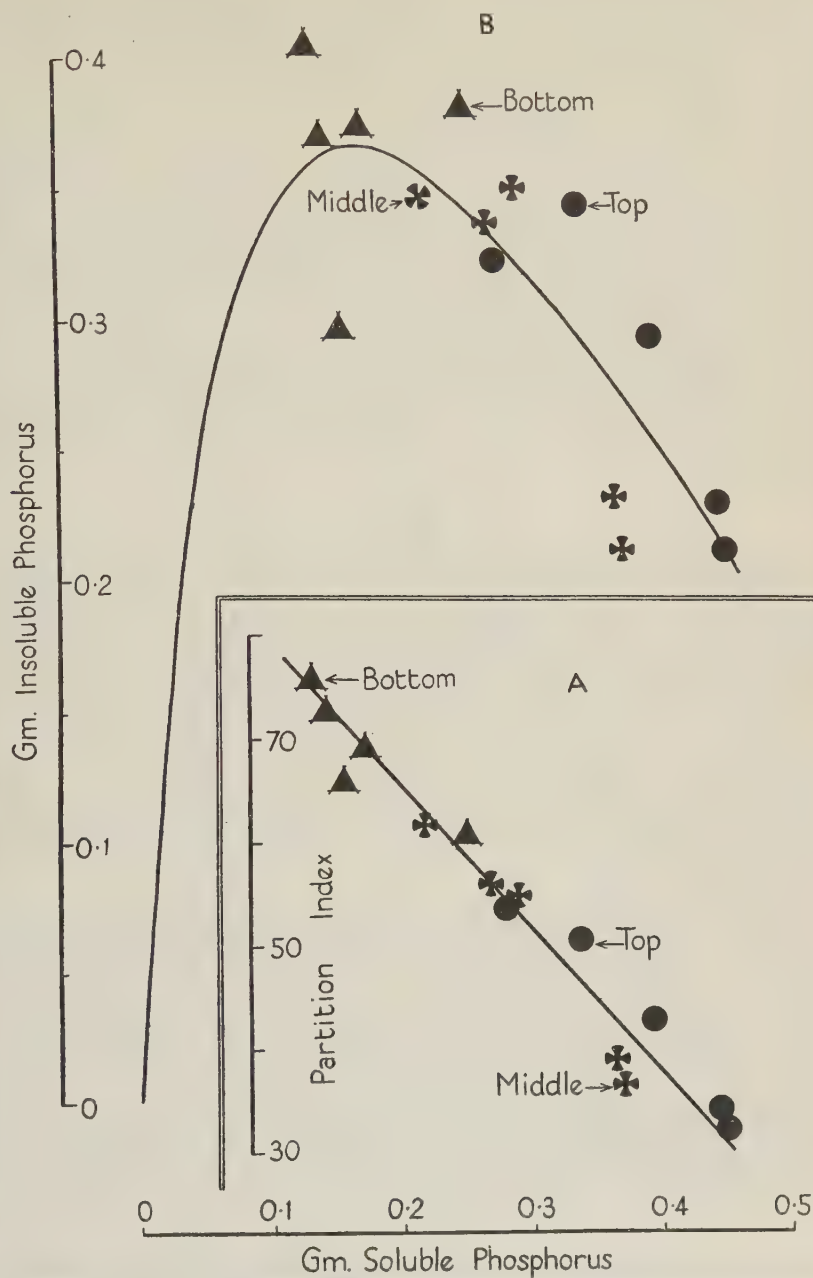
## IV. PHOSPHORUS

For the leaves of experiment 2 we also have data for soluble and insoluble phosphorus. We have calculated the partition index for phosphorus and



FIGS. 4 A and B. Fig. 4 A. The partition indices for nitrogen plotted against crystalloid-N per 100 gm. dry weight for 'Top', 'Middle', and 'Bottom' leaves of experiment 2. The regression lines of partition index on crystalloid-N for 'Top' and 'Bottom' leaves are also shown. Fig. 4 B. Protein-N plotted against crystalloid-N per 100 gm. dry weight, with the curve calculated from the regression line for all data of A.

plotted it against soluble phosphorus per 100 gm. dry weight in Fig. 5 A, using data for the three ages of leaves independently. Again the points tend to fall



FIGS. 5 A and B. Fig. 5 A. The partition indices for phosphorus plotted against soluble phosphorus per 100 gm. dry weight, with regression line of partition index on soluble phosphorus. Fig. 5 B. Insoluble phosphorus plotted against soluble phosphorus per 100 gm. dry weight with the curve calculated from the regression line of A.

on a straight line and give a very high negative correlation ( $-0.963$ ). The partition indices cover a wide range from 30 to 75 per cent. It would seem that for phosphorus as well as for nitrogen adsorption is the factor regulating the conversion of soluble to insoluble phosphorus and vice versa. The curve relating soluble to insoluble phosphorus in terms of dry weight is given in Fig. 5 B. This was calculated from the regression data between the partition indices and soluble phosphorus per 100 gm. dry weight. It will be noted that the experimental points all lie beyond the maximum where there is a negative correlation between soluble and insoluble phosphorus, while the corresponding points for nitrogen (see Fig. 4 B) all lie before the maximum, where there is a positive correlation between the soluble and insoluble fractions.

As far as can be judged from the available data, there does not appear to be any age effect in the case of phosphorus. The five points for each age do not lie on such straight lines as is the case for nitrogen, but there seems to be no doubt that over the whole series, soluble phosphorus is *the* controlling factor in partition.

#### V. LABILE CARBOHYDRATE

In experiment 4 there were in all eighteen treatments, and for each of these data for total (sap-soluble) sugars and for polysaccharides are available. Total carbohydrate, i.e. total sugars plus polysaccharides, has been termed labile carbohydrate (cf. Mason and Maskell, 1931), and in Fig. 6 (A) we show the partition indices for labile carbohydrate (polysaccharides as percents. of labile carbohydrate) plotted against total sugars per 100 gm. dry weight. Again it will be noticed that the points tend to lie on a straight line and show a very high negative correlation ( $r = -0.968$ ). The calculated curve relating total sugars to polysaccharides per 100 gm. dry weight is also shown (B). As in the case of phosphorus the experimental points lie beyond the maximum value for the insoluble fraction.

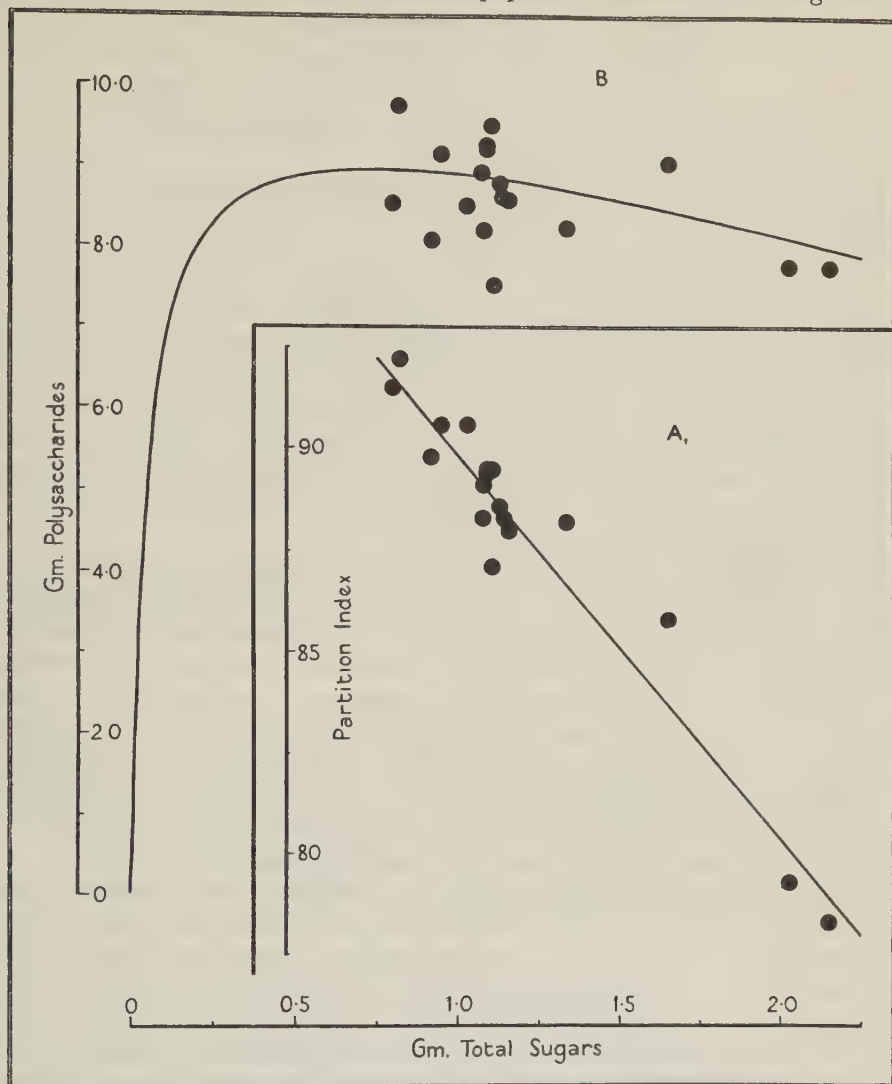
The close similarity between the polysaccharide-sugar relation and the protein-crystalloid-N relation suggests that they may both be controlled in a similar manner, i.e. that there may be a dominant adsorption factor controlling the polysaccharide-sugar equilibrium. We have tested this idea out on data from a diurnal experiment, but the relation does not hold, possibly because the changes are too rapid to allow of equilibrium being attained. In the present experiment the plants were placed in a dark house at dusk on the evening before collection and were collected without further exposure to light, thus allowing time for equilibrium to be attained.

#### VI. DISCUSSION

The outstanding feature of the work reported in this paper is the exactness of the relationship between protein and crystalloid-N. This is best shown by the straight-line relation between the partition indices and the concentrations of crystalloid-N per 100 gm. dry weight. Although the results for only four



experiments are given in this paper, we have checked the results of many other experiments over a very wide range of conditions. Thus all the results given by Phillis and Mason (1939) in the first paper of this series fit the regression



FIGS. 6 A and B. Fig. 6 A. The partition indices for labile carbohydrate plotted against total sugars per 100 gm. dry weight with regression line of partition index on total sugars. Fig. 6 B. Polysaccharides plotted against total sugars per 100 gm. dry weight with the curve calculated from the regression line of A.

line given in Fig. 3 very well indeed. For all these experiments, the correlation coefficient between the partition indices and the concentration of crystalloid-N per 100 gm. dry weight is  $-0.985$ . Plants grown in 6-hour light have

given data which fit on to the regression line of Fig. 3. The data available for plants grown in the field also fit in with the regression line. These available field data cover only a limited range of partition indices, however, and experiments covering a much wider range will be necessary before it can be safely concluded that a single regression line will adequately cover all data. All the data discussed in this paper refer to leaves from young plants still in the vegetative stage or just commencing to flower. What happens in older plants or other types of cotton is still uncertain.

It seems clear, however, that in vegetative plants the protein level in terms of dry weight is determined predominantly by the concentration of crystalloid-N. As the latter increases, protein increases at first rapidly, then more and more slowly until a point is reached where further increase in crystalloid-N causes an actual diminution in the amount of protein. It must be emphasized that the only other factor that we have found to affect the relation between protein and crystalloid-N is the age of the leaf. With increasing age the leaf requires an increasing crystalloid-N concentration to produce a standard protein level. As we have stressed, the relation between protein and crystalloid N is characteristic of apolar adsorption, and we have therefore suggested that adsorption of crystalloid-N plays a dominant role in regulating the equilibrium between protein and crystalloid-N.

The nature of the fractions constituting the crystalloid-N of the leaf is very imperfectly known. It is known, however, that the ratio of the components of crystalloid-N may fluctuate widely. Thus, asparagine (or glutamine) and nitrate are usually present at high levels when the crystalloid fraction is abundant. Nothing is known of the protein or proteins of the cotton leaf. It is at least surprising, in view of the heterogeneity of the crystalloid fractions, that this relation between protein and crystalloid-N should hold over such a wide range. It is possible that the crystalloid fractions are extremely labile and that both organic and inorganic nitrogen are involved in some form of equilibrium between the various crystalloid fractions.

We have also calculated the correlation coefficients between soluble phosphorus per 100 gm. dry weight and the partition indices for phosphorus for the experiments described in our previous paper (Phillis and Mason, 1939). It amounts to  $-0.869$ , a value not as high as we found for experiment 2 of the present paper, but still highly significant. It must be remembered that there are variable amounts of insoluble inorganic phosphorus in the leaf, and that the actual determination of phosphorus is not as accurate as that of nitrogen owing to possible losses in converting organic phosphorus to phosphate. A further difference between phosphorus and nitrogen which may have some significance is that normally 90 per cent. or more of the soluble phosphorus is phosphate whereas only 10 to 20 per cent. of the crystalloid-N is present as in organic nitrogen.

It is impossible to say much about the relation between polysaccharide and sugar, though here again, the observed relation is surprising in view of the

fact that 'soluble sugar' includes at least four different sugars (Phillis and Mason, 1933), while polysaccharides include starch and such hemicelluloses as are hydrolysed by normal hydrochloric acid.

## VII. SUMMARY

1. The problem of protein regulation in the leaf is considered.
2. In the first experiment it is shown that discs punched from leaves and floated on a nutrient solution containing inorganic nitrogen can form protein as readily as intact leaves. It is concluded that the synthesis of protein is determined by factors in the leaf and is independent of any factor exerted by the rest of the plant.
3. In four subsequent experiments covering a wide range of conditions it is shown that the protein-N level (protein-N per 100 gm. dry weight) is largely determined by the crystalloid-N level (crystalloid-N per 100 gm. dry weight). As the level of crystalloid-N is increased there is a rise in the protein-N level which reaches a maximum. After this, a further rise in the crystalloid-N level causes a decline in the protein-N level. It is pointed out that this type of relation is characteristic of apolar adsorption.
4. Examination of rather limited data on the relation between polysaccharides and total sugars, and between insoluble and soluble phosphorus, suggests that adsorption may also play an important part in determining the levels of polysaccharide and insoluble phosphorus respectively.

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# Physiological Studies in Plant Nutrition

## XIII. Experiments with Barley on Defoliation and Shading of the Ear in Relation to Sugar Metabolism

BY

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(From the Research Institute of Plant Physiology, Imperial College of Science and Technology,  
London)

With twelve Figures in the Text

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IN a recent paper (Archbold and Mukerjee, 1942) the view was expressed that sugars stored in the stems of barley plants were in no way essential to the development of the ear, and indeed it was doubtful if there was any upward movement of these sugars at all. This conclusion was based on studies of seasonal changes in the carbohydrates, in which loss of sugar from the stem was compared with the simultaneous increase of dry weight in the ear, and was supported by evidence obtained from unpublished experiments with defoliated plants and plants with shaded ears. The present paper describes in detail the latter experiments which were designed not only to examine the effects of the treatments on sugar metabolism, but also to determine the part played in assimilation by the stems and ears themselves. If stored sugar is not utilized as a source of starch, the carbohydrate stored in the grain must arise from material translocated immediately from the site of assimilation, and since starch storage proceeds actively during the later phases of the growth cycle, when leaves are senescent, it becomes a matter of some interest to ascertain the proportion of the grain yield attributable to direct assimilation by parts of the plant other than the leaves.

The experiments of Dehérain and Dupont (1901), Stålfelt (1935), Boonstra (1929), Smith (1933), and Watson and Norman (1939) have already shown that, in cereals, assimilation by the stems and by the ears themselves contributes substantially to the grain yield. For wheat both Boonstra and Smith found the contribution of the ear to be of the order of 30 per cent. and a similar figure for barley was found by Watson and Norman. Smith also estimated the contribution of the leaves and stems at 30 per cent. each. Boonstra's experiments included total and partial defoliations as well as shading of the ear, and from them he concludes that leaves below the uppermost pair contribute little or nothing to the carbohydrate of the wheat ear, while the total contribution of the leaf sheaths and stems appears to be as high as 40 per cent. He does not claim any great accuracy for these estimates, but regards the general conclusions as established, namely, that assimilation by the stems and ears plays a vital part in ear development and that lower leaves are ineffective as regards ear growth.

There are obvious objections to both defoliation and shading (see Watson and Norman, 1939) as a means of approach to the problem of the site of assimilation. Leaf removal may have injurious effects other than that of diminishing the assimilating surface, and the absence of light may equally have effects on ear growth in addition to the prevention of assimilation. That the effect of defoliation may be complicated by such secondary effects is indicated in Boonstra's experiments, where the contribution of each leaf was determined either by allowing only the leaf in question to remain on the plant or by removing this leaf alone. The apparent contribution was 30 per cent. higher in the latter case, but in the absence of duplicate values the significance of this difference cannot be assessed. The difficulties attending the procedure of maintaining the ears, leaves, &c., separately in the light in a carbon-dioxide-free atmosphere were too great, having regard to the size of the contemplated experiment; recourse to the alternative but less satisfactory method was thus inevitable. Within the limitations imposed by possible effects of treatment other than the prevention of assimilation, the contributions of the various organs to the carbohydrate of the ear have been estimated.

The data will be considered in two sections. First, analyses made at relatively short intervals (up to 35 days) after treatment, which record the effects, in equal times, of treatments given at four different stages of growth and are principally concerned with the problem of sugar metabolism; second, the data from the harvest collections which record the total effect of treatment on the yield and from which the contributions of the separate organs to the carbohydrates of the ear have been estimated.

#### EXPERIMENTAL PROCEDURE

Barley (Plumage Archer) was sown on April 29, 1940, in 10 in. glazed pots containing soil. There were 18 seeds sown in each of 250 pots and the number of plants was subsequently reduced to 6 per pot by thinning in two stages at

the emergence of the 2nd and 3rd leaves. At the time of the second thinning a dressing of 2 grammes of  $\text{NaNO}_3$  per pot was given. The soil used was, from choice, very deficient in nitrogen, so that even with the added nitrate the conditions were those of partial nitrogen deficiency. This limited the number of tillers per plant to 5.5 in the controls, so that the number of ears to be shaded was not too large, and further ensured that the concentration of sugar, whose response to treatment it was desired to study, should be high.

The shading and defoliation treatments, four in all, were begun on four separate occasions, using 60 pots chosen at random for each occasion. The first was begun at the time of emergence of the ear on the main axis (60 days after sowing), and the others followed at weekly intervals during the ensuing period of ear emergence and accumulation of sugar in the stem. The last treatment occasion coincided with the maximum sugar content of the stem. Each treatment occurred once in each pot, the plants being assigned at random, within the pots, to the various treatments. Together with the control this procedure utilized 5 out of the 6 plants in every pot. Samples were collected for analysis at intervals of 5, 17, and 35 days after treatment for each occasion, and final collections were made at the normal harvest time (19 weeks after sowing). The final collection was spread over 4 days.

The treatments given are set out below, and the letters there used to designate the five groups of plants will be used throughout to distinguish the different treatments. The term 'sheath' will be used to designate the flag-leaf sheath only, and the term 'stem' will include sheaths other than that of the flag-leaf. The term 'shading' refers to shading of the ear only.

- C. Untreated control.
- F. Complete defoliation, effected by removing with scissors all leaves at the junction with the sheath.
- Fs. Defoliation as above, together with removal of the flag-leaf sheath.
- E. Shading of the ear as described below.
- EFs. Combination of treatments E and Fs.

The treatments C, Fs, E, EFs were chosen to give a  $2 \times 2$  factorial arrangement with respect to ear shading and defoliation with sheath removal. Treatment Fs will afford a measure of the combined effect of leaves and sheaths, and treatment E that of the ears, by the differences from the control values, while treatment EFs will measure directly the contribution of the stem in the event of absence of interactions between the two other types of treatment. The factorial arrangement will moreover provide information as to the extent of any such interaction. In practice the estimate of the stem contribution was complicated by the effect of defoliation on the number of ears reaching maturity, as well as by the fact that treatment interactions, although small, were not entirely absent. These points are more fully discussed in the section dealing with the contribution of the plant organs to the ear dry weight. Comparison of the treatments F and Fs was designed to determine the

contribution of the sheath, since it was not possible to undertake the additional work required to include treatment F in a factorial scheme.

#### METHOD OF SHADING

The method of shading is shown diagrammatically in Fig. 1. The shades consisted of inner cylinders of black paper ( $6 \times 1\frac{1}{2}$  in.) supported within outer

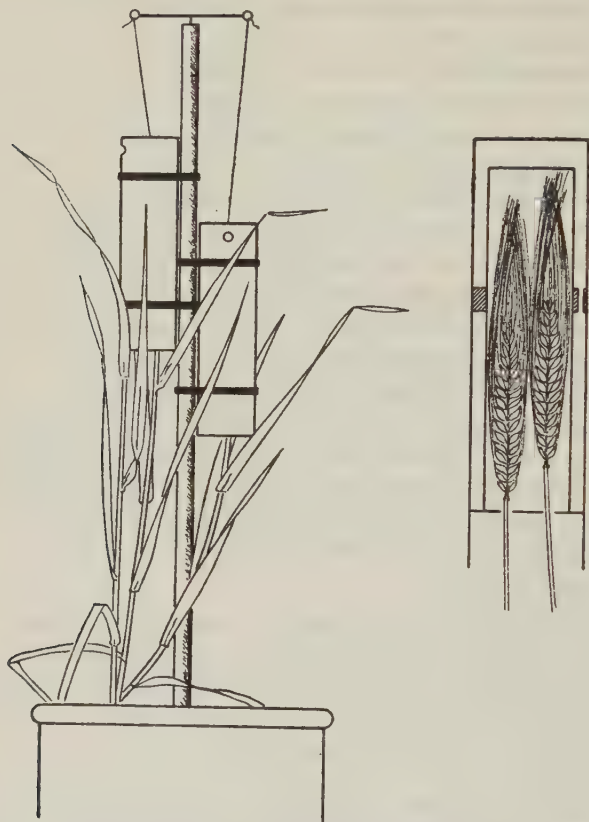


FIG. 1. Diagram illustrating the method of shading the ears; see text.

cylinders of white cartridge paper ( $7\frac{1}{2} \times 2$  in.) by means of cork washers. Two wedges were cut out of the washers to allow unrestricted passage of air in the space between the two paper cylinders, and two holes were punched near the top of the outer cylinder for ventilation purposes. A 2 in. white cardboard disc was fitted inside the white cylinder, thus closing the lower end of the inner black cylinder. Slits were cut in the discs to allow the peduncles of the shaded ears to pass through. The white cylinders were painted on the outside to render them reasonably weather-proof. The shades were attached by strings to a wire T-piece fastened into the top of a cane in each pot. A stout wire connected the T-pieces in each row of pots to stakes in the ground



at the end of the rows, thus preventing any violent movement of the canes in windy weather. The strings permitted adjustment of the height of the shade to that of the ear to be covered. When the ears were inserted, the shade was held against the cane by two rubber bands, and the plant also carefully tied to the cane. These bands were gradually replaced by string ties as the rubber perished, but the use of the rubber bands greatly facilitated the application of the shades. Each shade was designed to cover two ears, but only ears of nearly the same height were inserted in the same shade. Since 2 plants in each pot (E and EFs) received shading treatment, 4 to 8 shades were required for each pot according to number and heights of the ear-bearing tillers.

On the first occasion of treatment only the ears on the main axis were emerging; these were carefully pulled out of the sheaths and shaded. Subsequently the plants were examined frequently (daily as far as possible) and each ear shaded as it reached the same stage of emergence. The shaded ears of this group were thus never exposed to full light. By the fourth occasion most of the ears had emerged, so that the process of shading could be carried out almost completely at one time. Complete exclusion of light is not claimed, as some trouble was experienced in keeping the basal cardboard discs in place, and when these became displaced temporarily some light was reflected up into the bottom of the black cylinders. Furthermore, in a few cases the emergence of an individual ear was overlooked for a few days since daily examination was not feasible at the busiest time. These delays in shading and the short periods when a little light penetrated the shades are not considered to have resulted in serious errors in the estimates of the effects of the various organs.

Duplicate samples of plants from each treatment group and of controls were collected at each analysis date. The samples consisted of one plant from each of 5 pots making 10 samples of 5 plants each at each collection. Sampling was begun at 9 a.m., and as the operation required about 4 hours the order in which the 10 samples were dealt with was randomized. Since 5 pots contributed to each replicate 40 pots were required for the four sampling dates for each occasion. The additional pots allowed provided samples for determining the initial composition of the plants at each of the four treatment occasions, and also made possible rejection of pots in which the ears were damaged by shading or in which diseased plants appeared.

For analysis the plants were divided into leaves, flag-leaf sheaths, stems, and ears. The number of ears and the fresh weight of each part was recorded. Aliquot samples were then weighed out for determination of dry weights and total sugar content. The subsequent procedure was that already described in detail (Archbold, 1938, 1942). The total sugar estimated was the sum of glucose, fructose, sucrose, and fructosan, and the residual material remaining after the extraction of the sugar was dried and weighed and is recorded as residual dry weight. It is the fraction insoluble in alcohol and cold water and

consists mainly of polysaccharides and proteins. It was not possible to separate the grain from the rest of the ear at the time of sampling, but this was subsequently done for the residual dry material of the harvest collections. At this time the residual dry weight constitutes over 90 per cent. of the total dry weight, so that satisfactory comparative measures of grain weight were obtained.

It may be noted here that the defoliated plants tended to ripen off more quickly than those retaining their leaves, and also that the ears after prolonged shading lost all trace of green colour, but the awns were frequently tinged with pink, and more rarely quite brightly tipped with crimson. This colouring matter did not appear to be due to anthocyan either free or in glucoside form, as it was not removed either by water or alcohol.

#### EFFECTS OF SHADING AND DEFOLIATION IN THE 35 DAYS FOLLOWING THE APPLICATION OF TREATMENT

The shading and defoliation treatments were given on four occasions 60, 67, 74, and 81 days after sowing, and analyses were carried out 5, 17, and 35 days after each of these dates. The stages of the growth cycle covered by these 35-day periods are shown in Fig. 2 by markings along the growth curves constructed from the data for the control plants and representing dry weights of the aerial parts and of the ears alone. During the 3 weeks between the first and the fourth treatment-occasion growth rate of the aerial parts remained approximately constant near the maximum value, but that of the ears increased rapidly. In both cases the 35-day intervals include some part of the phase of falling growth rate preceding the attainment of the maximum values for dry weight. It is obvious therefore that the growth increments made by the control plants in equal times following the beginning of the treatment of the experimental plants will not be equal, except for the first 5-day period, which for all four occasions falls on the approximately linear part of the growth curve. Between the 12th and 35th days after treatment growth increment for the first two occasions will be considerably greater than that for the last two. Furthermore, the mean values for dry weight, &c., for each treatment occasion will increase from the first to the fourth since the initial values are higher at the later dates. The leaves and sheaths reached maximum dry-weight values 75 days after sowing, about the time of the third treatment, and the stems after 90 days, a little later than the fourth treatment.

The times of treatment were chosen so that the level of sugar increased from the first occasion to the fourth. The lowest level was that occurring at the emergence of the ear on the main axis, this being manifestly the earliest time at which shading of the ears could be begun. If the primary assimilate is used preferentially for growth and only the excess appears as stable sugar, then restriction or cessation of the normal sugar accumulation should be observed in the groups of plants treated on the first two occasions, while utilization of stored sugar to make good, even in part, the deficiency in the supply of

assimilate due to treatment should result in rapid loss of sugar from the plants treated on the last two occasions.

The data for the 12 collections are presented in Tables I, II, III, and IV.

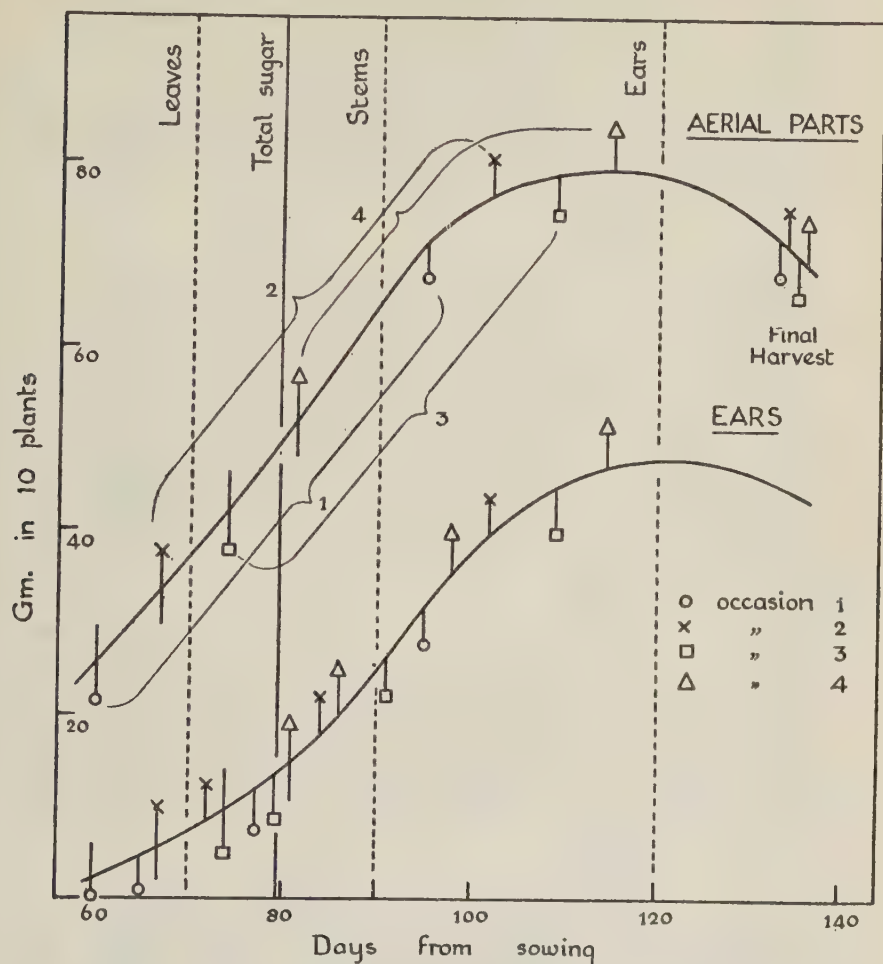


FIG. 2. Growth curves (dry weights) of the aerial parts and of the ears of barley showing treatment and analysis dates.

On the upper curve the 35-day periods following each of the four treatment occasions are marked, together with the final harvest dates and on the lower the analysis dates 5, 17, and 35 days after each treatment occasion. The positions of the maximum dry weights of the separate organs are shown by dotted lines and of the maximum total sugar in the aerial parts by an unbroken line.

Duplicate values for dry weight, residual dry weight, total sugar, and the number of tillers bearing ears are given, together with the treatment totals for the whole series. The percentages of the maximum dry weight attained by each organ at the time of treatment are also included. These percentage





TABLE II

*Residual Dry Weight (gm.) of Samples (5 Plants) of Barley receiving Shading and Defoliation Treatments on Four Occasions. Analysed at Intervals of 5, 17, and 35 Days after Treatment. Treatments as in Table I*

Sowing date: 29 April 1940.

Occasion and days after sowing.	Analysis date (days after treatment).	Leaves			Sheaths			% of max. present at treatment.			Stems			% of max. present at treatment.			Ears		
		C	E	% of max. present at treatment.	C	F	E	C	F	E	C	F	E	C	F	E	C	F	E
I (60)	5	2.17	2.84	93	0.80	0.67	0.80	4.73	4.51	5.54	4.51	5.54	6.42	4.73	4.51	5.54	1.53	1.09	1.48
	17	2.09	2.11		1.07	0.84	0.94	5.93	5.50	4.86	5.93	5.50	5.01	5.03	4.77	4.43	2.03	1.77	1.43
	35	2.38	2.50		1.02	0.86	1.65	7.02	4.97	5.30	7.02	4.97	8.95	3.35	2.90	2.68	3.35	2.90	2.68
		2.07	2.02		1.16	0.85	1.24	7.35	5.21	6.22	7.35	5.21	8.09	3.27	2.76	2.44	3.27	2.76	2.44
		2.47	1.94		1.30	1.07	1.41	8.68	7.51	6.32	8.68	7.51	7.44	12.54	10.95	9.32	12.54	10.95	9.32
					1.75	1.03	1.76	9.88	5.96	5.32	9.88	5.96	8.39	14.69	10.09	7.96	14.69	10.09	7.96
II (67)	5	2.72	2.57	100	1.52	1.12	1.13	6.34	6.39	5.98	6.34	6.39	6.71	2.30	2.71	2.70	2.30	2.71	2.70
	17	2.55	2.50		1.09	1.28	1.02	5.37	7.30	4.77	5.37	7.30	6.45	3.07	2.87	1.84	3.07	2.87	1.84
	35	1.96	2.69		1.08	1.26	1.31	8.05	8.51	6.04	8.05	8.51	6.09	5.80	6.35	5.56	5.80	6.35	5.56
		2.71	2.64		1.44	0.90	1.40	8.13	5.95	7.10	8.13	5.95	6.71	7.10	4.85	6.06	7.10	4.85	6.06
		2.27	2.44		1.68	1.24	1.43	7.78	7.09	6.41	7.78	7.09	6.07	17.10	15.70	11.42	17.10	15.70	11.42
		2.44	2.34		1.60	1.14	1.57	10.11	6.14	7.24	10.11	6.14	5.71	18.68	13.53	15.16	18.68	13.53	15.16
III (74)	5	1.92	2.27	falling	1.11	1.34	1.43	5.54	7.59	7.04	5.54	7.59	7.31	3.53	4.61	4.65	3.53	4.61	4.65
	17	2.22	2.34		1.38	1.15	1.34	7.24	6.93	7.95	7.24	6.93	8.63	5.63	4.66	4.79	5.63	4.66	4.79
	35	2.08	2.41		1.82	1.34	1.48	11.29	9.00	9.24	11.29	9.00	6.18	12.50	10.85	12.95	12.50	10.85	12.95
		2.38	2.38		1.59	1.22	1.39	8.72	7.82	7.36	8.72	7.82	7.45	10.26	10.42	9.97	10.26	10.42	9.97
		2.21	2.05		1.54	1.20	1.34	9.60	6.94	6.73	9.60	6.94	6.25	23.62	17.73	17.12	23.62	17.73	17.12
		2.40	2.10		1.49	1.18	1.47	7.93	6.93	7.38	7.93	6.93	7.20	22.04	16.46	16.64	22.04	16.46	16.64
IV (81)	5	2.10	2.04	falling	1.26	1.07	1.22	7.29	6.91	10.01	7.29	6.91	7.53	6.69	7.15	8.44	6.69	7.15	8.44
	17	2.11	2.31		1.15	1.07	1.18	7.62	6.94	8.76	7.62	6.94	9.35	7.96	7.95	9.02	7.96	7.95	9.02
	35	2.31	2.16		1.54	1.05	1.45	9.24	6.08	8.80	9.24	6.08	7.97	15.99	12.33	15.97	15.99	12.33	15.97
		2.24	2.04		1.37	1.31	1.26	8.36	7.65	6.19	8.36	7.65	7.73	14.80	13.79	12.51	14.80	13.79	12.51
		2.01	2.29		1.21	1.07	1.54	7.12	5.89	6.51	7.12	5.89	7.12	17.68	16.31	13.02	17.68	16.31	13.02
		2.33	1.63		1.74	1.43	1.20	9.49	7.76	7.17	9.49	7.76	6.70	23.70	21.93	13.99	23.70	21.93	13.99
Treatment totals		55.63	55.76		32.71	26.69	31.96	189.61	162.58	164.44	189.61	162.58	161.83	255.95	218.86	215.56	255.95	218.86	215.56
Standard errors of differences between totals		1.70	—		1.24	—	—	5.86	—	—	5.86	—	—	10.01	—	—	10.01	—	—
Significant differences } 5% level		—	—		2.55	—	—	12.07	—	—	12.07	—	—	20.66	—	—	20.66	—	—
Significant differences } 1% level		—	—		3.47	—	—	10.40	—	—	10.40	—	—	28.02	—	—	28.02	—	—



TABLE IV

*Number of Tillers bearing Ears in Samples (5 Plants) of Barley receiving Shading and Defoliation Treatments on Four Occasions, at Intervals of 5, 17, and 35 Days after Treatment. Treatments as in Table I*

Sowing date: 29 April 1940.

Occasion and days from sowing.	Analysis date (days after treatment).	C	F	Fs	E	EFs
I (60)	5	13	9	10	12	16
		14	12	15	12	10
	17	16	15	13	23	18
		20	13	15	20	12
	35	21	16	19	24	18
		30	18	17	24	15
Totals		114	83	89	115	89
II (67)	5	16	16	16	17	14
		20	20	12	14	15
	17	19	19	16	20	19
		22	14	18	21	16
	35	26	20	17	23	19
		24	17	20	28	19
Totals		127	106	99	123	102
III (74)	5	15	20	17	22	17
		19	16	21	20	18
	17	30	25	23	24	18
		24	20	18	20	22
	35	31	21	22	27	19
		28	22	22	26	18
Totals		147	124	123	139	112
IV (81)	5	18	17	21	18	21
		21	18	21	20	23
	17	29	18	24	27	23
		27	24	20	22	22
	35	29	19	18	32	22
		24	21	22	21	20
Totals		148	117	126	140	131
Treatment totals		536	430	437	517	434

Standard error of differences between treatment totals, 21.4.

“ “ “ “ “ “ for each occasion, 10.7.

Significant differences (5% level)  $\left\{ \begin{array}{l} n = 24 \quad 44.2. \\ n = 6 \quad 26.2. \end{array} \right.$

values were calculated from appropriate values read off smooth curves drawn through running means of the observations for three successive dates for the control data. These curves were published in the previous paper (Archbold and Mukerjee, 1942). Analyses of variance have been carried out on all the data, and the results will be discussed with reference to the results of this statistical analysis.

Inspection of the treatment totals for dry weight (Table I) shows that shading was without effect on the average dry weight of leaves and sheaths, but resulted in a reduction of the dry weight of stems and ears, while defoliation reduced the dry weight of all three remaining components, sheaths, stems, and ears. Comparing the defoliation treatment (F) with that in which the flag-leaf sheath was removed as well (Fs), it is found that sheath removal exerted no additional effect on the stem dry weights, but tended to further slight reduction in that of the ears. Although in the case of the average dry weight this difference does not reach the significance level, it will be shown in the sequel that the sheath does in fact exert an effect on the dry weight of the ear. Since there is no treatment effect on the leaves, and that on the sheaths is confined to the defoliation treatment, the principal effects to be dealt with will be those on the stems and ears. The data of Table I show that the ears continue to increase in dry weight throughout the 35-day periods for all four occasions, although treatment restricts growth in all cases. The maximum dry weight of the stems was reached about 90 days from sowing, so that the stem dry weight increased throughout the 35-day period at occasion 1, which ended at day 95, but on the three later occasions stem dry weight was falling at the end of the 35 days. The time sequence of the rise and fall of dry weight is little affected by treatment, but as with the ears the amount of growth is restricted. As an example of the progress of dry-weight accumulation the data for stems and ears for occasion 1 are shown in Fig. 3, where the sums of the duplicate values (i.e.  $g$  in 10 plants) in Table I are plotted against time. The initial values of dry weight (not recorded in the table) and the harvest values (see Table VII) are also included.

In this case the increase in dry weight of the stem was much reduced but not completely prevented by defoliation alone, and there is little difference between the three treatments involving defoliation. Shading the ears reduced the stem dry weight to a less degree than defoliation. In the ear the restriction in growth was about the same for defoliation and shading, but the removal of the sheath as well as the leaves resulted in a further reduction of the dry weight, and when both treatments E and Fs were applied simultaneously ear growth was very limited. The curves for the other three occasions are similar in type to that of Fig. 3, but the effects, especially of defoliation, tend to be smaller when treatment is applied at a later stage of growth (see Fig. 4).

The effects produced by treatment on the average residual dry weights (Table II) are substantially the same as those for total dry weight, except in



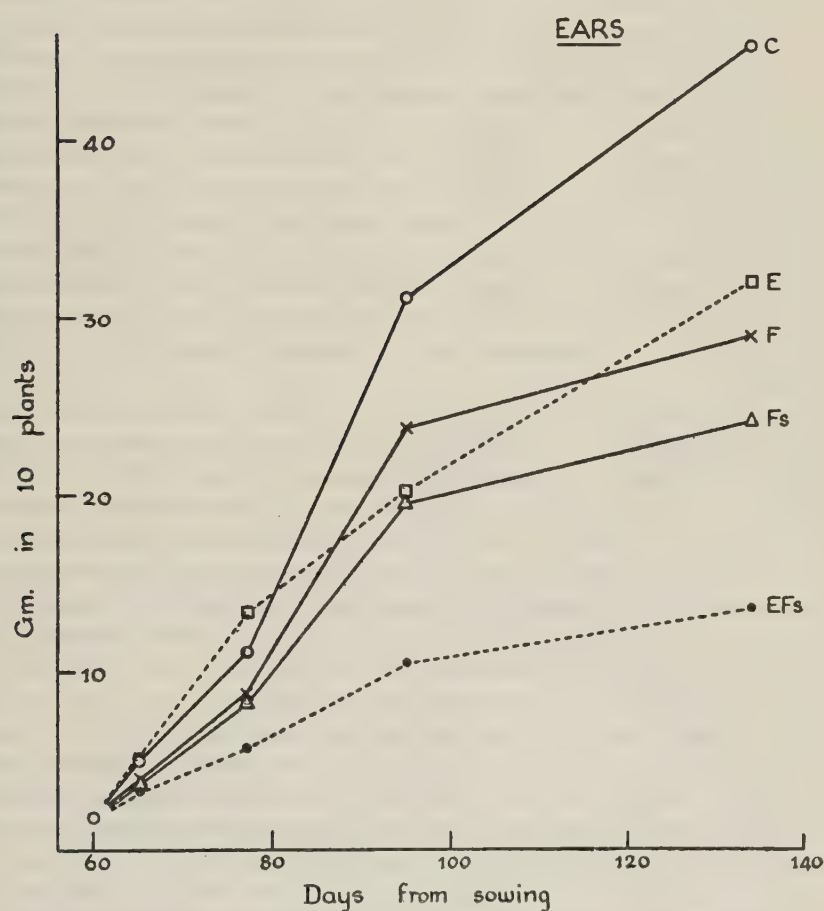
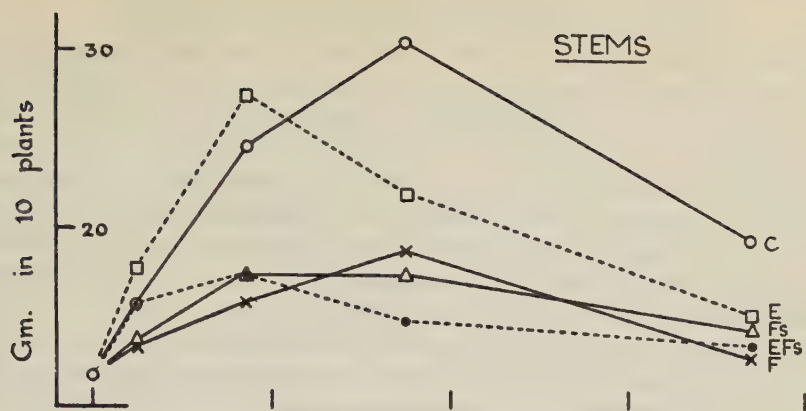


FIG. 3. Progress of accumulation of dry weight in stems and ears of barley receiving ear-shading and defoliation treatments 60 days after sowing (treatment occasion 1) and harvested 135 days after sowing. C, control; F, leaves removed; Fs, leaves and flag-leaf sheath removed; E, ears shaded; EFs, combination of E and Fs.

the case of the stems of plants with shaded ears, where the difference due to this treatment is not significant, indicating that the observed difference in total dry weight must be in the soluble fraction and that structural components are not affected.

Mean sugar contents (Table III) are lowered throughout by all treatments, and all the differences, save that between the sugar contents of the leaves of control and ear-shaded plants, are significant. The reduction due to defoliation is greater than that due to shading, the difference between the effects of the two types of treatment being least in the ears.

Since in no case was any significant difference found in the mean values for the three constituents measured, for the leaves of the shaded and control plants, these organs need little further consideration. It should, however, be pointed out that the absence of a mean effect is not due to opposing effects on the different occasions, as can readily be seen from the separate treatment totals for each occasion given in Table V. It seems therefore safe to conclude that any effect of shading the ear on leaf metabolism is very small. On the first treatment occasion (60 days after sowing) the leaves had already nearly reached their maximum dry weight, so that total leaf dry weight was falling throughout the major part of the experimental period. This fall represents the balance between loss from the older leaves and gain in the production of new leaves on later tillers. Neither the losses of leaf dry weight from mature leaves nor the production of leaves on later tillers appear, therefore, to be affected when the ears are shaded, and in particular it may be emphasized that the leaf sugar was not depleted by the demands of ears unable themselves to photosynthesize.

The average effects of defoliation and of shading the ear are not widely different with respect to the total yield of dry matter in the ear, but the data of Table IV recording the numbers of ears produced reveal that the two effects are exerted in dissimilar ways. Thus the effect of shading on the number of ears produced is small and not significant, while all the three treatments involving defoliation reduce the number of ears reaching maturity, and to the same extent. The chief effect of defoliation was therefore to prevent the development of immature tillers and so to reduce the final yield, while the chief effect of shading the ears was to reduce the size of the individual ears without affecting the normal production of later tillers.

The treatment totals for each of the four occasions, for the constituents dry weight, residual dry weight, and total sugar are given in Table V. In the upper section are the sums of the groups of six observations recorded in Tables I, II, and III, that is, the amounts of each constituent in 30 plants; and in the lower section sums of six similar figures expressed in terms of the weight of each constituent per stem, which may be calculated from the data of Tables I to IV. The extent to which the reduction in ear number accounts for the lower yield of defoliated plants may be judged by comparing columns C and F in the lower section of Table V. For the ears the dry weights and

residual dry weights were consistently higher in the treated than in the untreated plants, the mean difference reaching the level of significance, while the sugar levels were lower in treated plants; there is therefore little treatment effect when the adjustment for reduction in number of ears has been made. Per unit stem the residual dry weights are again higher in the defoliated plants, but dry weight and sugar levels are lower, the latter significantly so. Residual dry weights per unit sheath were unaffected, while dry weight and sugar were again lower than in the control. Total dry-weight reduction due to defoliation, other than that accounted for by reduction in ear number, is thus confined to the soluble fraction of the dry weights in all parts of the plant, and is adequately covered by the lower sugar values. On the whole the differences between the sugar levels are greater than those between dry weights, so that there is some indication that material which normally would have appeared as sugar has in the defoliated plants been otherwise utilized.

It cannot be concluded that the higher values for dry weight of ears in the defoliated plants indicate a real increase over those of the controls. The average values for the control plants include the later tillers, which produce smaller ears than those on the main axis and the one or two earliest formed tillers, whereas by inhibition of tiller development in defoliated plants the ear-bearing tillers are restricted to those normally producing larger ears; consequently a high average value per ear will result even if defoliation is without further effect. A similar argument naturally applies to the stems and sheaths. The data do, however, show that if there is any reduction in size of the ear it is very small when defoliation is carried out 60 days after sowing or later. In general, therefore, the defoliated plants produced normal ears but fewer in number than control plants, and structural development of stems bearing these ears was unaffected, though less sugar accumulated in them. It is reasonable to assume from the above evidence that the leaves were ineffective in providing a substrate for filling the grain, but were of paramount importance in the formation of the ears. The material for grain-filling must have been supplied entirely by sheaths, stems, and the ears themselves.

The part played by the leaves in grain-filling may possibly be determined by the conditions of growth, since leaves do under some circumstances contribute, but it is likely, as Boonstra (1929) found for wheat, that only the uppermost leaves are effective in this respect. In a previous experiment (Archbold, 1938*a*) with plants receiving abundant nitrogen, and having large flag-leaves compared with those of the present experiment, the size of the ears was somewhat restricted by defoliation. The treatment was carried out at a slightly earlier stage of growth than in the present instance, so that in the absence of further observations it is impossible to discriminate with certainty between the effect of time of treatment and the conditions of growth.

Before discussing in detail the relation of treatment effects to the time of their application a comparison may be made between the effects of simple defoliation and of defoliation accompanied by sheath removal. It will be

TABLE V

*Dry Weights, Residual Dry Weights, and Total Sugar in Barley Plants receiving Shading and Defoliation Treatments (Treatments as in Table I). Figures are Treatment Totals of Six Observations made after Each of Four Occasions on which Treatment was applied, and are derived from Tables I-IV. Significant Differences (5% level) are shown below Each Set of Observations: A for Treatment Totals ( $n = 24$ ) and B for Individual Occasions ( $n = 6$ )*

Treatment.	Leaves.			Sheaths.			Stems.					Ears.				
	C		E	C	F	E	C	F	Fs	E	EFs	C	F	Fs	E	EFs
	g. per sample of 30 plants.															
Occasion I	23.27	24.76	11.72	7.64	12.61	71.04	48.16	48.91	67.20	48.21	36.54	31.98	47.25	36.54	31.98	38.62
" II	23.93	23.36	12.66	9.87	11.85	73.91	38.13	34.03	64.12	53.42	56.54	50.88	68.21	56.54	50.88	50.88
" III	21.00	20.29	13.53	10.37	12.32	74.57	31.13	31.13	60.40	58.38	77.29	70.20	88.95	77.29	70.20	68.38
" IV	20.33	19.05	11.36	9.48	10.78	67.35	38.95	64.80	61.54	60.21	90.11	92.15	98.36	90.11	92.15	73.94
Total .	89.43	87.46	49.27	37.38	47.56	286.87	228.37	231.92	259.26	220.22	260.48	252.84	302.77	260.48	252.84	231.82
Sig. diff. A.	6.2			3.6			18.6					20.6				
" " B.	3.7			2.1			10.4					12.3				
Occasion I	14.07	14.56	7.10	5.32	7.80	43.59	33.56	33.56	45.20	33.60	28.67	25.77	37.41	28.67	25.77	27.98
" II	14.65	15.18	8.41	6.94	7.86	46.58	41.38	37.54	44.91	38.50	46.01	42.74	54.14	46.01	42.74	39.68
" III	13.81	13.55	8.93	7.43	8.45	50.32	45.21	45.90	47.17	43.02	77.58	64.73	77.58	64.73	66.12	55.97
" IV	13.10	12.47	8.27	7.00	7.35	49.12	42.33	47.44	45.26	46.71	86.82	80.87	86.82	79.45	80.87	64.82
Total .	55.63	55.76	32.71	26.69	31.96	189.61	162.58	164.44	181.34	161.83	218.86	215.50	255.95	218.86	215.50	188.45
Sig. diff. A.	3.6			2.6			12.1					20.7				
" " B.	2.1			1.5			7.2					12.3				
Occasion I	3.34	3.53	3.47	1.19	3.23	20.08	9.55	8.48	16.87	5.63	4.35	3.19	6.71	4.35	3.19	5.30
" II	2.64	2.55	3.14	1.41	2.66	20.08	9.69	9.53	13.24	6.74	4.69	3.52	6.19	4.69	3.52	4.54
" III	1.78	1.60	3.03	2.01	2.21	16.45	11.29	12.30	12.74	8.23	4.83	5.00	6.05	4.83	5.00	4.80
" IV	1.36	1.15	1.97	1.33	1.50	11.50	8.56	9.61	10.14	7.34	3.79	3.97	4.28	3.79	3.97	3.41
Total .	9.12	8.83	11.61	5.94	9.60	68.11	39.09	39.02	52.99	27.94	17.66	15.68	23.23	17.66	15.68	18.05
Sig. diff. A.	0.64			1.08			4.63					1.58				
" " B.	0.38			0.64			2.75					0.94				



Treatment.	Leaves.		Sheaths.			Stems.					Ears.				
	C	E	C	F	E	C	F	F <sub>s</sub>	E	EF <sub>s</sub>	C	F	F <sub>s</sub>	E	EF <sub>s</sub>
g. per sample of 6 stems.															
Dry weight.															
Occasion I	—	—	0.63	0.56	0.67	3.74	3.53	3.39	3.67	3.39	2.25	2.40	2.02	1.88	1.31
" II	—	—	0.62	0.56	0.59	3.56	3.30	3.30	3.23	3.24	3.01	3.17	2.90	2.35	2.01
" III	—	—	0.58	0.51	0.54	3.14	3.00	3.17	2.94	3.15	3.45	3.68	3.74	2.88	2.88
" IV	—	—	0.47	0.49	0.41	2.80	3.02	3.07	2.73	2.75	3.88	4.56	4.37	3.20	3.15
Total	—	—	2.30	2.12	2.28	13.25	12.94	12.93	12.57	12.53	12.59	13.81	13.03	10.31	9.35
Sig. diff. A.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
" " B.	.	.	.	0.14	0.09	.	.	1.06	.	.	.	.	0.85	.	.
				0.09				0.64					0.51		
Residual dry weight.															
Occasion I	—	—	0.38	0.31	0.41	2.33	2.49	2.34	2.44	2.35	1.72	1.86	1.60	1.35	1.02
" II	—	—	0.40	0.39	0.39	2.22	2.35	2.28	2.24	2.32	2.36	2.57	2.43	1.81	1.61
" III	—	—	0.37	0.36	0.37	2.08	2.21	2.25	2.07	2.32	2.02	2.07	3.15	2.34	2.34
" IV	—	—	0.34	0.36	0.34	2.03	2.18	2.22	2.00	2.13	3.44	4.02	3.84	2.79	2.75
Total	—	—	1.49	1.50	1.51	8.66	9.23	9.09	8.75	9.12	10.44	11.52	11.02	9.29	7.72
Sig. diff. A.	.	.	.	0.13	0.08	.	.	0.74	.	.	.	.	0.92	.	.
" " B.	.	.	.	0.08		.	.	0.44		.	.	.	0.55		.
Total sugar.															
Occasion I	—	—	0.102	0.008	0.171	1.06	0.68	0.57	0.92	0.40	0.356	0.305	0.218	0.274	0.126
" II	—	—	0.158	0.081	0.141	0.90	0.55	0.60	0.69	0.42	0.300	0.270	0.218	0.236	0.150
" III	—	—	0.139	0.102	0.101	0.74	0.37	0.63	0.58	0.45	0.278	0.244	0.252	0.271	0.189
" IV	—	—	0.086	0.071	0.071	0.50	0.45	0.45	0.45	0.33	0.182	0.108	0.185	0.158	0.119
Total	—	—	0.575	0.342	0.484	3.29	2.25	2.25	2.65	1.00	1.116	1.017	0.873	0.885	0.584
Sig. diff. A.	.	.	.	0.04	0.03	.	.	.	.	.	.	.	.	.	.
" " B.	.	.	.	.	.	.	.	0.25	.	.	.	.	0.08	.	.
" " C.	.	.	.	.	.	.	.	0.15	.	.	.	.	0.05	.	.

remembered that defoliation alone is not included in the factorial arrangement designed to test whether shading the ear and defoliation are independent in their effects and to examine the interactions between treatment and time of application.

It is obvious that the reduction in ear number necessitates reduction in stems and sheaths as well as ears, and it has already been pointed out that sheath removal does not further affect the average values of dry weights, residual dry weights, or total sugars in the stems. This is also true for the values for each separate occasion, as may be seen by comparing columns F and Fs, for stems, in Table V. The reduction in these constituents in the stem when leaves and sheaths are both removed may therefore be entirely attributed to leaf removal over the whole range of the experiment. For the ears, however, the totals for the separate occasions (Table V) reveal that the differences indicated by the average values of dry weight, &c., between the treatments F and Fs are confined to the first two occasions, the effects after the later treatments being quite insignificant. Unfortunately the sampling errors, mainly due to variability in the size of the plants, are large, and thus the effect of the sheath cannot be demonstrated with an acceptable degree of certainty by comparison of the results for the two earlier treatments alone. The values for the Fs treatment are consistently lower than those for the F treatment, for the first two occasions, but the differences in sugar content alone reach the level of significance, although the mean difference between the values for the dry weights per ear approaches the 5 per cent. level. Further evidence will be presented on this point when the interactions and the data of the harvest collections are discussed (p. 517), sufficient it is believed to warrant the conclusion that the sheath does contribute to the filling of the ear. The effects of defoliation alone on the sheath itself are also shown in Table V. As with the stems all the values are lower than in control plants, when expressed as weights per plant, largely owing to the reduction in ear number. If weights per stem are considered, no difference is found in residual dry weight, but dry weights and sugar contents are lowered, the loss in the former being again entirely accounted for by the sugar loss. The effect is therefore similar to that of defoliation on the stems, namely sugar level is somewhat reduced, but structural components are not affected in the sheaths on tillers reaching maturity.

The interactions between the two types of treatment (ear shading and defoliation), and between the time of application and the treatment effects, are shown diagrammatically in Figs. 4 to 9 using the method of plotting devised by Richards (1941). Each diagram represents a  $4 \times 2 \times 2$  factorial arrangement, namely two treatments (shading and defoliation with sheath removal), each at two levels (present or absent), combined with the four occasions at which the treatments were given. The points represent the sums of the values of either dry weight, residual dry weight, or total sugar for each occasion and are plotted on the appropriate ordinates in the usual way. Each

point is thus a sum of 6 values, namely duplicate observations on each of 3 analysis dates after treatment, and there are 16 sums for every diagram (data of Table V). The sums are inserted in 6 groups at equal intervals along the abscissa in the following order: C<sub>1</sub>; C<sub>2</sub>, E<sub>1</sub>, F<sub>s1</sub>; C<sub>3</sub>, E<sub>2</sub>, F<sub>s2</sub>, EF<sub>s1</sub>; C<sub>4</sub>, E<sub>3</sub>, F<sub>s3</sub>, EF<sub>s2</sub>; E<sub>4</sub>, F<sub>s4</sub>, EF<sub>s3</sub>; EF<sub>s4</sub>, where the numbers represent the four treatment occasions, and the letters the treatments as set out on p. 489. The 16 points are joined by 28 lines falling into two main groups. One group comprises 12 single lines, whose slopes represent the differences between the values for each treatment on the various occasions, and the other group is of 16 double lines with slopes representing the differences, at each occasion, between the totals of treatment values. Of these double lines the pied show differences due to defoliation and the white those due to shading. There are four quadrilaterals bounded by white and pied lines from which the interactions between treatments may be judged, that is to say the effects of defoliation with sheath removal, and of shading the ear, either alone or in combination. Twelve further quadrilaterals bounded by two single and two double lines (white or pied) show the interaction effects of treatments with the time of their application. The interaction represented by any one quadrilateral approaches zero as the quadrilateral approaches a parallelogram.<sup>1</sup> The main effects of treatment already mentioned are of course also readily discerned by inspection of the diagrams.

In Fig. 4 are shown the total dry weights of the ears (sums of the values for each occasion) in terms of the dry weight in 10 plants, upper diagram, and in terms of the dry weight per ear, lower diagram. In both diagrams the consistent upward slope of the line C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub> indicates the progressive increase in the size of the plants between the treatment occasions (see also Fig. 2). In terms of dry weight in 10 plants both types of treatment lower the dry-weight yield on all occasions. At the first occasion the F<sub>s</sub> effect is greater than that of E, at the second occasion the two effects are equal, and on the third and fourth occasions shading had the greater effect. These differences between the treatment effects are brought about, as the treatment date advances, both by decreasing effects of F<sub>s</sub> and by increasing effects of E, as is shown by the convergence of the lines C<sub>1-4</sub> and F<sub>s1-4</sub>, and of E<sub>1-4</sub> and EF<sub>s1-4</sub> and divergence of the lines C<sub>1-4</sub> and E<sub>1-4</sub> and of F<sub>s1-4</sub> and EF<sub>s1-4</sub>. These effects constitute the interaction between the time of application and treatment. The numerical differences between the values for samples with and without shaded ears, and with and without defoliation and sheath removal on the four occasions, calculated from the data in Table V, are:

Differences due to treatment E:	20·88,	32·83,	44·85,	47·88		
„	„	F <sub>s</sub> :	34·16,	33·47,	25·21,	11·46.

For treatment E the total interaction with occasion is significant at the

<sup>1</sup> This brief account is taken as nearly as is appropriate from Richards' own description. His paper should be consulted for details of the more extended use of this type of diagram.

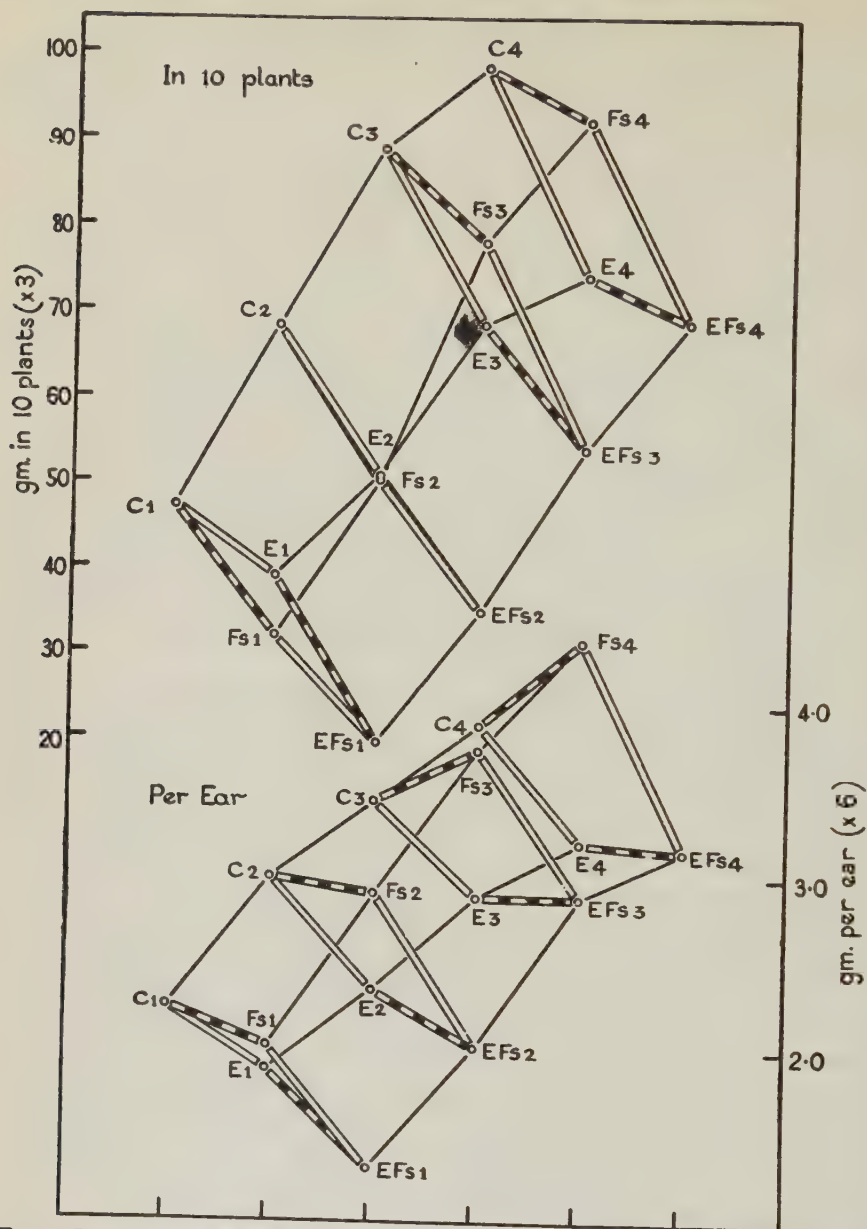


FIG. 4. Interaction diagram of a  $4 \times 2 \times 2$  arrangement showing the effect, during 35 days following treatment, of defoliation with flag-leaf sheath removal and of shading the ear at four stages of growth, on the dry weight of the ears of barley. Points represent sums of duplicate observations for three analysis dates (see Table V). C, control; Fs, defoliated and flag-leaf sheath removed; E, ears shaded; EFs, combination of E and Fs. Numbers represent the four treatment occasions (O).

Significant interactions (per plant)  $E \times O$ ,  $Fs \times O$ ; (per ear)  $Fs \times O$ ,  $E \times Fs$ .



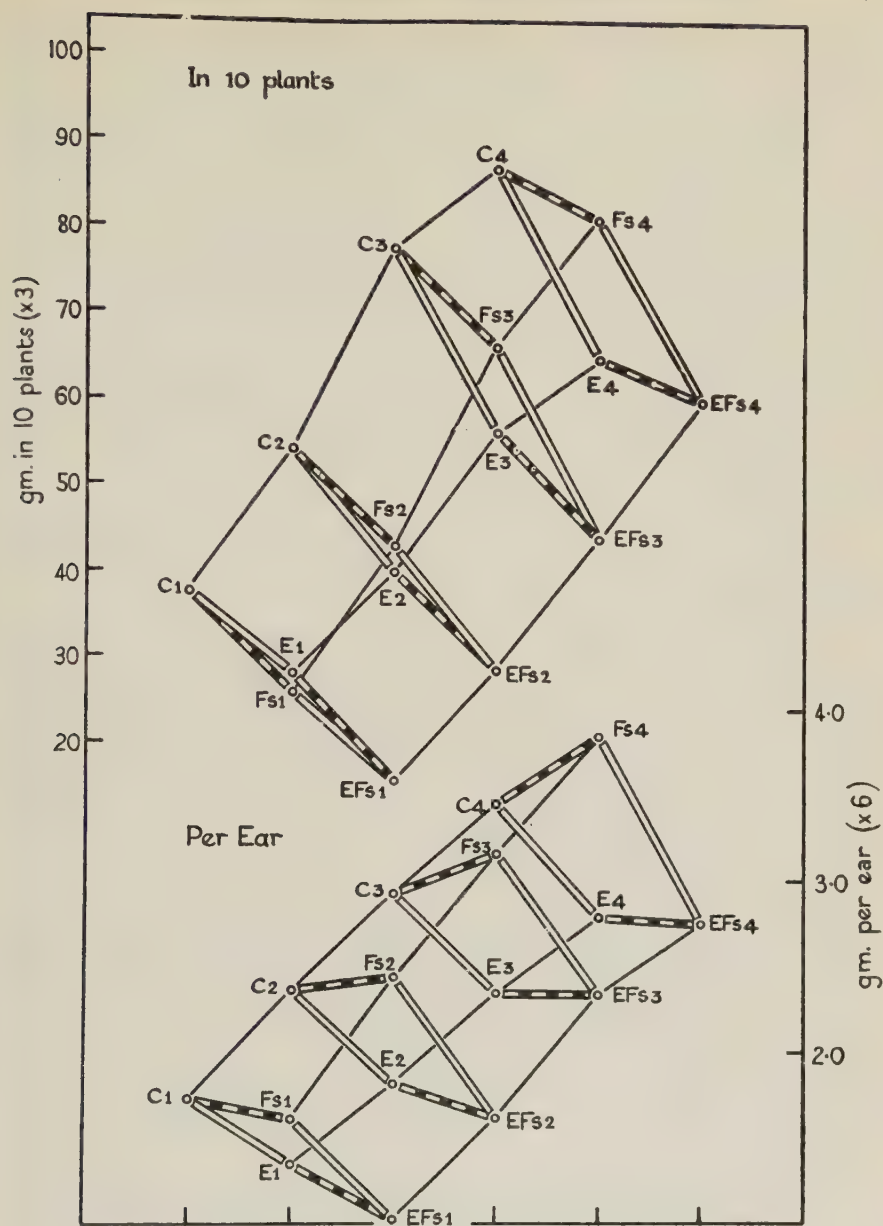


FIG. 5. The effect of treatment on the residual dry weights of the ears.  
Treatments and arrangement as in Fig. 4.

Significant interactions (per plant)  $E \times O$ ; (per ear)  $Fs \times O$ ,  $E \times Fs$ .

5 per cent. level, and if the components of the interaction (3 degrees of freedom) are considered separately as described by Yates (1937), the difference attributable to the linear component is significant at the 1 per cent. level. For the treatment Fs the total interaction does not reach the level of significance, but the linear component of interaction is significant at the 5 per cent. level. It has already been shown that the principal effect of defoliation is to reduce the number of ears produced, so that the interaction between Fs and occasion may in part be attributed to the fact that a higher proportion of the total number of ears is already differentiated at the later treatment dates as compared with the earlier. Similarly, the increasing effect of shading over the 35-day periods under consideration is due to the larger number of ears available for shading at occasion 4 than at occasion 1. Presumably the assimilation of the ear will increase as it grows to its full size, so that during the period of ear emergence total assimilation due to the ears will for a time increase as later ears emerge.

The sides of the quadrilaterals  $C_1$ ,  $E_1$ ,  $Fs_1$ ,  $EFs_1$ ;  $C_2$ ,  $E_2$ , &c., which represent the interaction between E and Fs, in no case depart significantly from parallelism. There is thus no indication in Fig. 4 (upper diagram) of interaction between treatments.

In terms of the dry weights per ear (lower diagram) the Fs treatment slightly lowers the dry weight on the first two occasions, but on the second two the values for treated plants are higher than those of the controls. The interaction effects between treatment and occasion are thus similar to those already found above, but are more pronounced and are here significant at the 1 per cent. level. There is therefore a diminishing effect of this treatment with later dates of application. It will be recalled (Table V) that defoliation alone resulted in dry weights per ear greater than those of the control throughout, so that sheath removal on the first two occasions must be deemed to have effected the reduction in ear size during the subsequent 35 days. The effect of Fs therefore diminishes with the later treatment dates both because of the smaller reduction in ear number and because the effect of the sheath in reducing dry weight becomes less pronounced. The interaction between shading and occasion is not significant when dry weight is expressed in terms of the weight per ear, so that the effect on total dry weight in this case is due solely to the increase in the number of ears available for shading at the later dates.

In the lower diagram of Fig. 4 the quadrilaterals bounded by white and pied lines are in no case parallelograms, indicating treatment interactions in the sense that the effect of shading is greater in the presence of defoliation than in its absence. This result may, in part, be explained by the fact that all the ears on the defoliated plants are large and therefore give a higher average value for the loss per ear due to shading, on the assumption that the shading effect is proportional to the size of the ear. If this were the sole reason for the observed interaction between these treatments, then the total

shading effect in the presence of defoliation (upper diagram) should be less than in its absence, since there are fewer ears to be shaded after defoliation treatment, while ear number is unaffected by shading alone (Table IV). There is, however, no suggestion of such interaction, as has already been stated; in fact the differences between the total dry weights of the ears in the shaded and not shaded groups in the presence and absence of defoliation are not even in the direction to be expected. It must therefore be concluded that there is a direct physiological cause for at least part of the interaction effect. Since the shading effect is greater in the absence of leaves and sheaths, it might be suggested that ear growth is restricted for lack of some factor supplied by these organs, but which the ear when in the light can provide for itself. The nature of such a factor is at present quite unknown.

Diagrams for residual dry weight similar to those discussed above are shown in Fig. 5. They resemble in all respects those of Fig. 4, and require no further comment beyond the evidence they provide that the major part of the effect of treatment on ear dry weight resides in the insoluble fraction, principally starch.

In Fig. 6 the data for dry weights of the stems are shown. The position is here somewhat different from that of the ears where increase in weight was continuous throughout the whole range of the experiment. In the stems phases of both increasing and decreasing dry weight have to be considered. The slopes of the lines C<sub>1</sub>, C<sub>2</sub>, &c., show that in the controls there was only a slight increase in the average dry weight up to occasion 3 and a marked fall by occasion 4. By reference to Fig. 2 and Table I it will be seen that for occasion 1, when 40 per cent. of the maximal stem dry weight had been attained before treatment, dry weight increased throughout the subsequent 35 days; at occasion 2, when 60 per cent. was initially present, growth in the control continued for only 23 out of the 35 days following treatment, while at occasion 4 the stems were all but fully grown at the outset and dry weight fell throughout the 35 days experimental period. The upper diagram of Fig. 6 shows plainly that there is a large effect of defoliation (Fs) on the first two occasions, covering the period of mainly-increasing dry weight in the control, with much smaller effects on the last two occasions when dry weight is on the whole falling. It is obvious from the convergence of the C and Fs lines and the E and EFs lines that there is a highly significant interaction between the defoliation treatment and the time of its application for the stems as well as the ears. The effect of shading is small throughout, and there is no significant interaction between this treatment and its time of application. In the lower diagram, where the effect is adjusted for ear number, it is found that the average values for the controls fall consistently, from occasion 1 to occasion 4, an expression both of the fact that the later tillers developing with the progress of time are smaller than those developing earlier and that dry weight is falling for a greater proportion of the 35 days on the later occasions. The effect of Fs is still to reduce the dry weights

after the first two occasions, but for the last two the effect is reversed, and the average values for treated plants are higher than those for the controls.

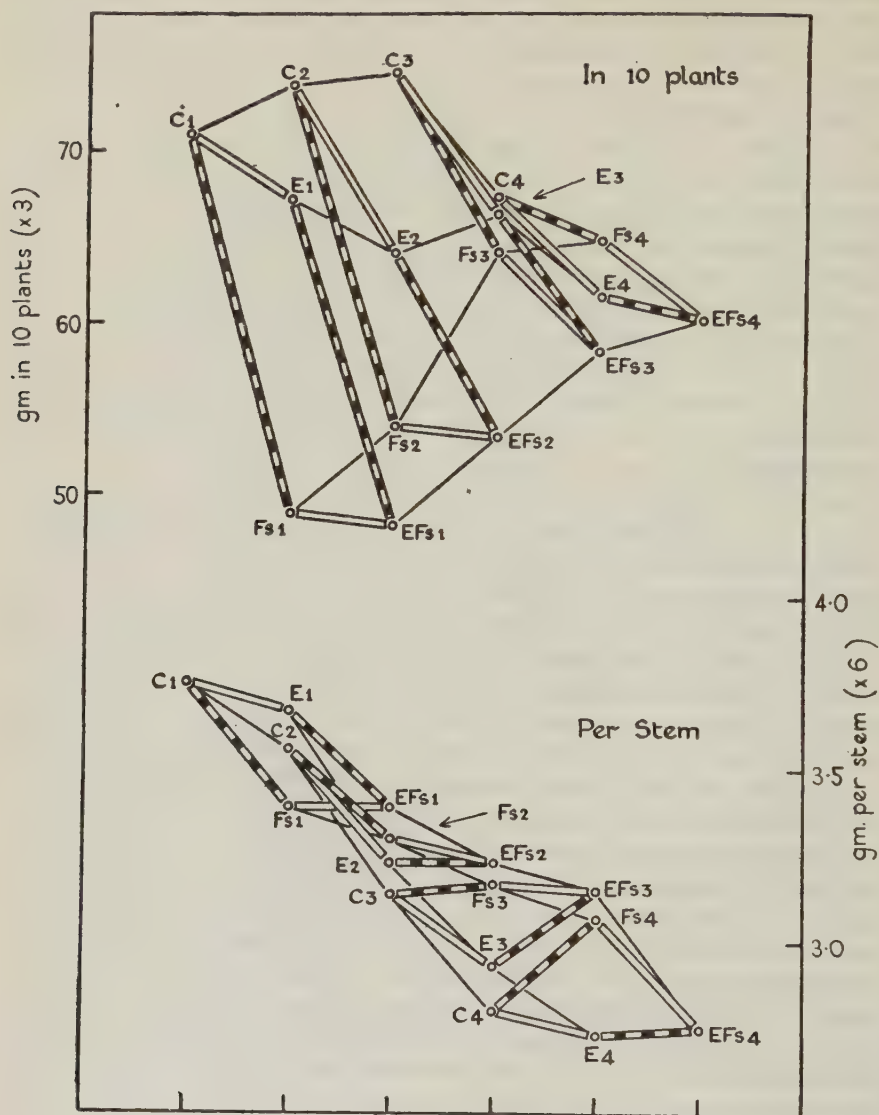


FIG. 6. The effect of treatment on the dry weight of the stems.  
Treatments and arrangement as in Fig. 4.

Significant interactions (per plant)  $F_s \times O$ ; (per stem)  $F_s \times O$ .

As in the case of the ears therefore any reduction in dry weight is not at this latter stage sufficient to offset the higher average values obtained from stems restricted to the large main axis and early tillers. Since dry weight is



falling during the greater part of the 35-day periods at occasions 3 and 4, it appears that defoliation has little or no effect on the losses of stem dry weight, but it affects the increase at the earlier stages of growth represented on occasions 1 and 2 both as regards the number of stems produced and

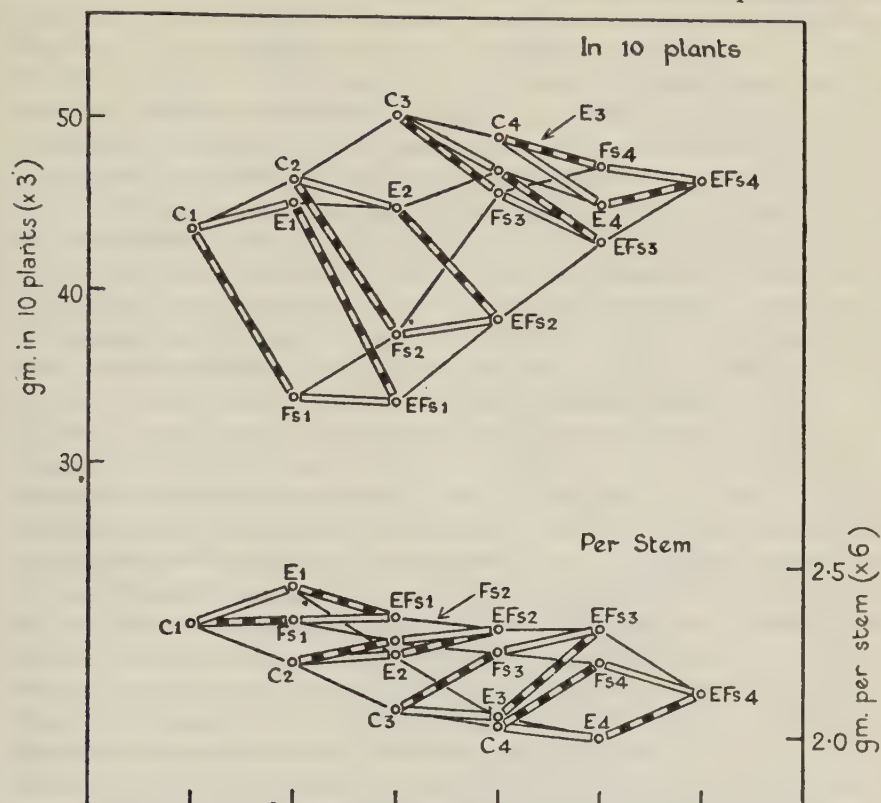


FIG. 7. The effect of treatment on the residual dry weight of the stems. Treatments and arrangement as in Fig. 4.

Significant interactions (per plant)  $Fs \times O$ ; (per stem) none.

the total dry weight per stem. It may at once be concluded that plants defoliated when the stem sugar is at its maximum do not utilize that sugar more rapidly than untreated plants, a conclusion borne out by the sugar values themselves (see p. 512).

The interactions between the two types of treatment are not significant whichever way the results are expressed, although there is some suggestion that the effects of shading may be less in the presence than in the absence of defoliation, an opposite effect to that suggested by the data for the ears.

In Fig. 7 the corresponding diagrams for the residual dry weights are given. The data for the weights of 10 plants (upper diagram) show the same trends as those for total dry weight as regards defoliation and sheath removal,

but the effects are considerably smaller showing that the reduction of residual dry weight in the case of the stems forms a smaller proportion of the total reduction than in the case of the ears. For ear shading the effects are no longer significant, so that the whole reduction in dry weight here falls on the soluble fraction for all occasions. Treatment interactions are again insignificant. The residual dry weight per stem (lower diagram) is little affected by either treatment as is shown by the approach to the horizontal of the double lines both white and pied. The  $F_s$  values tend to be higher than those of the controls in the later treatments, as already noted for the total dry weight. The only treatment effect on the residual dry weight of the stems is therefore the reduction necessarily accompanying the lower ear number in defoliated plants; all additional effects on total dry weight must thus be on the soluble fraction. Structural tissues of the stems which continue to develop at all are therefore unaffected by either treatment, at whatever time it is applied.

Diagrams for total sugar for ears and stems are given in Figs. 8 and 9. In both organs sugar content rises until the 79th day and then falls, so that phases of both rising and falling sugar are included, similar to those discussed for dry weight; it will be seen that the treatment effects follow the same general lines, namely large effects of defoliation on the first two occasions and smaller but still significant effects for shading, while for the two later occasions the  $F_s$  effect is reduced to about the same value as that of shading. The changes in stem sugar are much greater in magnitude than those of the ears, and there is no interaction between the treatments themselves. The type of effect is the same whether the data are expressed as weights per 10 plants or per ear and stem; so the differences due to defoliation are not here confined to those necessarily accompanying the reduction in ear number but indicate a lower level of sugar as well. The dry-weight differences already attributed to soluble constituents would thus appear to be largely accounted for by these differences in sugar content. In all four diagrams the interaction between  $F_s$  and occasion is again shown by the converging C and  $F_s$  lines, while there is no interaction between E and occasion. The convergence is brought about by a steep fall in the C and E lines together with a tendency to rise in the  $F_s$  and  $EF_s$  lines. The sugar values thus confirm the conclusion arrived at from the study of the dry-weight data, namely that there is no evidence of rapid sugar consumption when assimilating organs are prevented from carrying on photosynthesis, but there is a restriction of the accumulation of sugar as compared with untreated plants. Thus the effects of treatment on sugar content are large during the phase of increasing sugar content when the initial value is low, and small when sugar level is initially high and might be expected to fall rapidly to supply the demands of the plant deprived of the normal sources of carbohydrate. This treatment effect may be seen more clearly by the inspection of the data for the separate analysis dates in Table III, and is also shown in Fig. 10, where the data for stems at occasions 1 and 4 (as weights per stem) are plotted.

For occasion 1 (upper diagram) there is a highly significant interaction between treatment Fs and analysis date, the effect at A<sub>3</sub> being large compared with either that at A<sub>2</sub> or A<sub>4</sub>. This large difference at A<sub>3</sub> is clearly brought about by a large accumulation of sugar in the stems of control plants, and since there is at the same time a small increase in sugar in the plants of the Fs group there can be no question of the difference arising as a result of rapid consumption of stored sugar. Sugar accumulation is presumably prevented by the immediate utilization of the available assimilate for growth. Between A<sub>3</sub> and A<sub>4</sub> sugar level falls both in controls and treated plants, but the slope of the line C<sub>3</sub>, C<sub>4</sub> as compared with that of Fs<sub>3</sub>, Fs<sub>4</sub> shows that in fact sugar loss in controls was actually greater than that in treated plants at this stage. Shading the ear also restricts sugar accumulation but much less so than defoliation, so that the shaded ears are not even able to draw upon all the sugar being newly formed and stored in the stems and sugar continues to accumulate to some extent. Between A<sub>3</sub> and A<sub>4</sub> when sugar level is falling the difference from the controls does not increase with the E treatment, so that again it is found that consumption is unaffected by treatment, and differences in sugar level arise during the phase of increasing sugar content. For occasion 4 (lower diagram), when sugar is at its maximum value at the outset, the treatment effects have almost disappeared. At A<sub>2</sub> there is a small reduction due to treatment, equal for E and Fs, and probably due to continued accumulation of sugar in late developing tillers in the controls which delays a little the onset of the maximum rate of fall. This difference tends to disappear by A<sub>4</sub>. Furthermore, all four single lines representing differences between analysis dates are remarkably parallel, leaving no doubt that sugar is being lost at practically equal rates by both treated and control plants, and therefore rate of loss is independent of treatment. Intermediate situations to those shown for occasions 1 and 4 occur at occasions 2 and 3, the interaction between treatment and analysis date becoming less as the proportion of the total time diminishes during which sugar content is rising.

Considering the results so far discussed it becomes evident that ear-shading and defoliation treatments affect sugar level only in parts of the plant other than the ear itself. There is no breakdown of cellulose complexes to replace assimilation deficiencies; in fact residual dry weights are quite unaffected by conditions which supposedly create a large demand for carbohydrate. The sugar levels must therefore be the simple resultant of production and utilization of primary assimilates, and are not complicated by the supply of sugar from storage reserves. In untreated plants production is in excess of demand during stem elongation and sugar accumulates; this accumulation can be largely prevented by defoliation and sheath removal, but only to a more limited extent by shading the ear. In the ear itself normal development proceeds in a reduced number of ears in the absence of leaves and sheaths, and this is presumably brought about by utilization of all available assimilate and results in the failure of sugar to accumulate. When the ears are shaded,



however, starch storage is diminished, but the ear is unable to utilize more than a small fraction of the sugar still being formed in the stems. It would

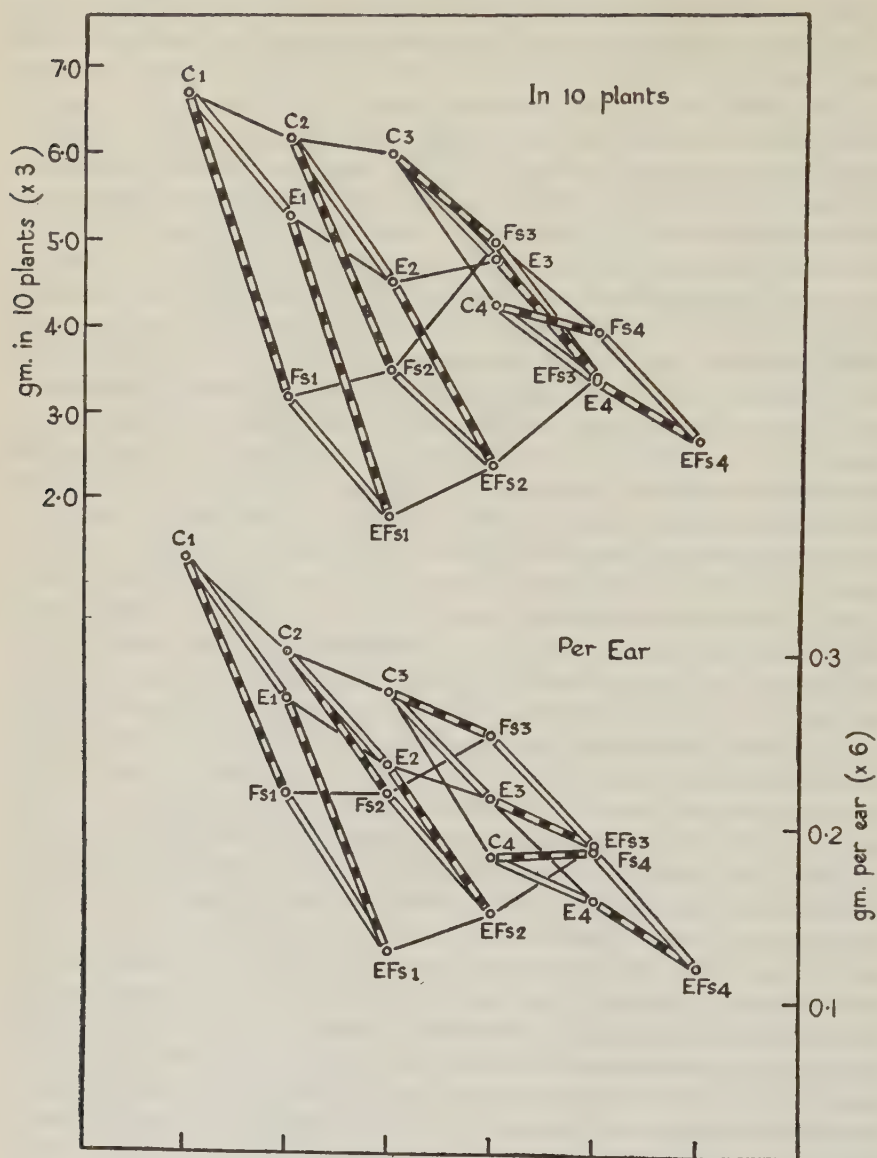


FIG. 8. The effect of treatment on the total sugars of the ears.  
Treatments and arrangement as in Fig. 4.

Significant interactions (per plant)  $Fs \times O$ ; (per ear)  $E \times O$  and  $Fs \times O$ .

appear therefore that under the conditions of the present experiment leaves supplied sugar in excess of requirements during the stage of stem elongation,



but this sugar was not subsequently available as a precursor of starch in the

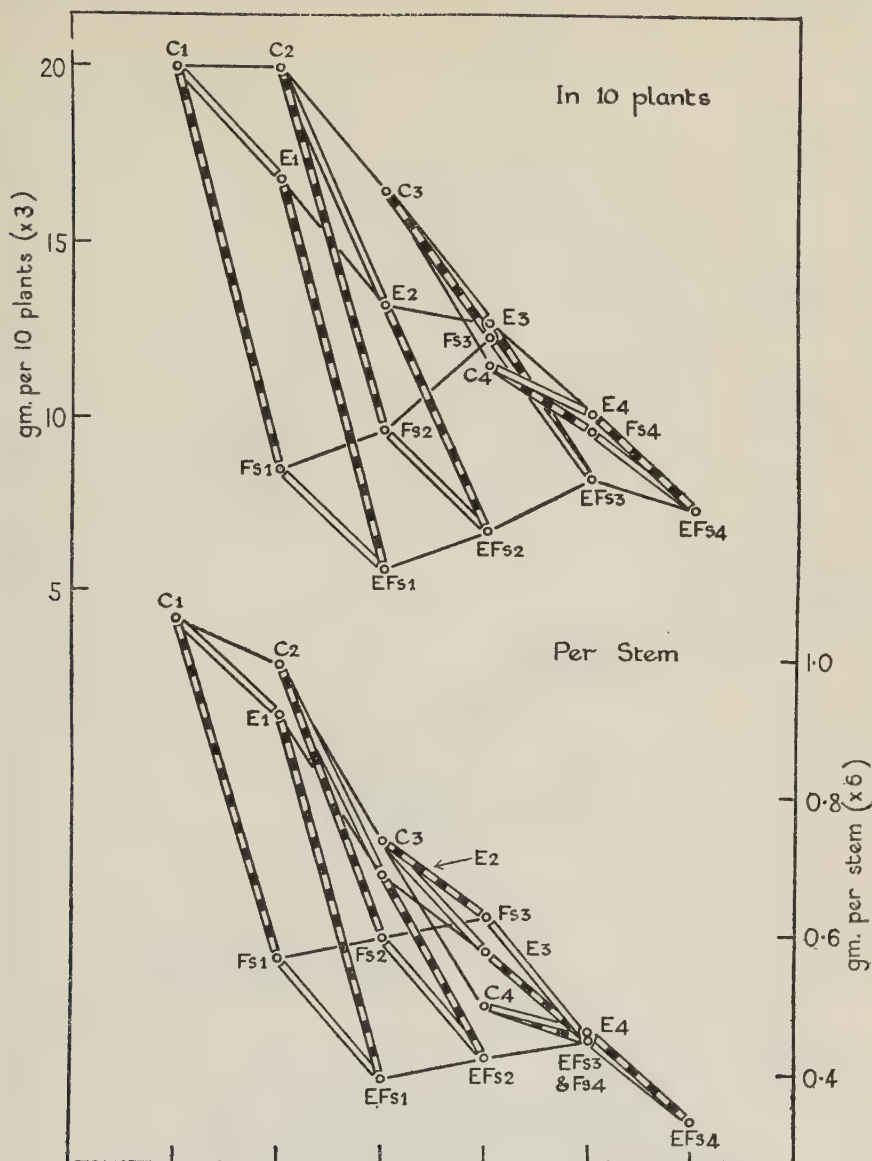


FIG. 9. The effect of treatment on the total sugars of the stems.

Treatments and arrangement as in Fig. 4.

Significant interactions (per plant)  $Fs \times O$ ; (per stem)  $F \times O$ .

ear. The present data do not afford evidence as to which parts of the stem are active in supplying assimilate to the ears, but since shading effects such as a small reduction in sugar level it may be supposed that the region on which

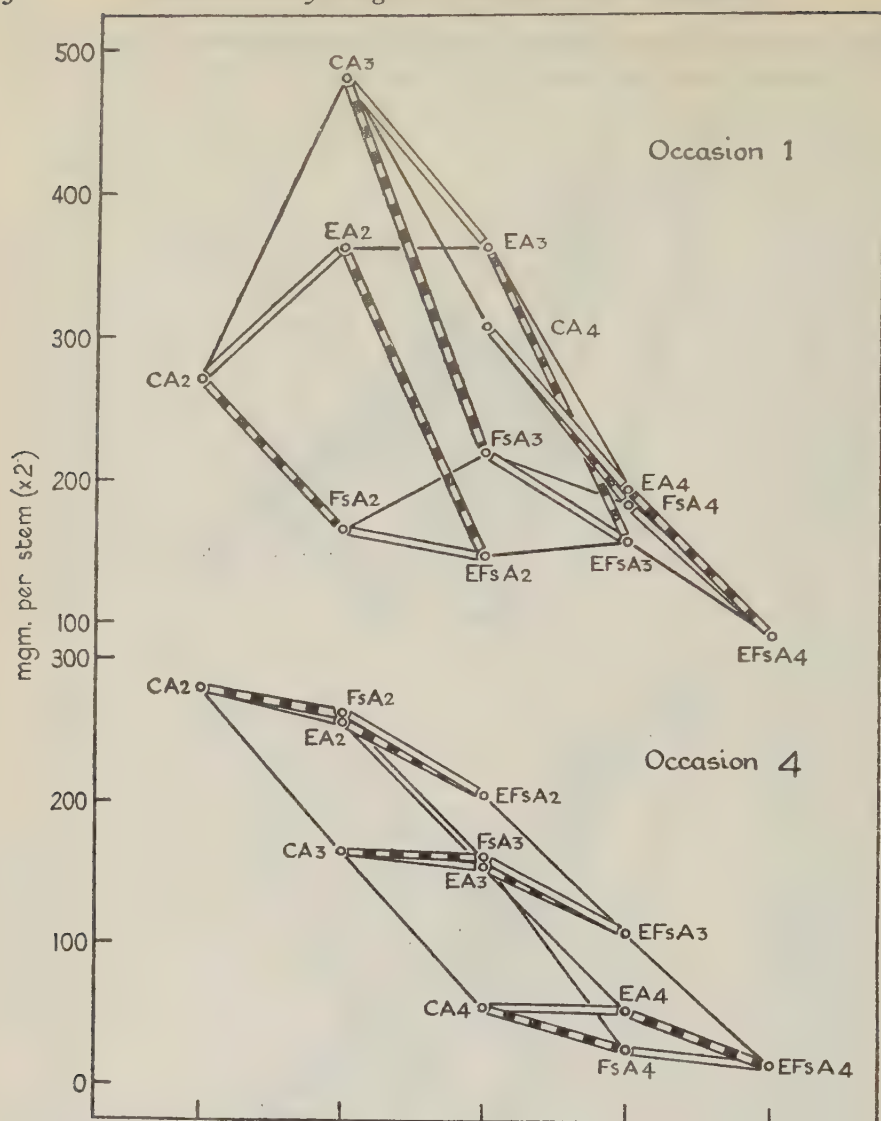


FIG. 10. The effect of treatment on the total sugar per stem, at intervals of 5, 17, and 35 days (A<sub>2</sub>, A<sub>3</sub>, A<sub>4</sub>) after application, for occasions 1 and 4.

Treatments as in Fig. 4, arrangement  $3 \times 2 \times 2$ .

Significant interactions, occasion 1,  $Fs \times A$ ; occasion 4, none.

the ear can draw is circumscribed and is likely to be the large top internode and the peduncle.

#### EFFECT OF TREATMENT ON THE PLANTS AT HARVEST

The data for the collections made at the normal harvesting time (135 days after sowing) are presented in Tables VI and VII. Table VI gives the dry

weights, residual dry weights, and total sugars for the various parts of plants treated on the four occasions, and Table VII the number of ears produced, the dry weights per ear, and the residual dry weights of 100 grains and of awns associated with 100 grains. The treatment totals are included in each case. A cursory examination of the data reveals that the total effects of treatment are similar to those already discussed in detail for the 35-day periods, namely a reduction in the number of ears, but not in their size, as a result of defoliation, and a reduction in size but not in number by shading of the ear. The sugar values in the ears have all now reached very low values and there is no longer any difference due to shading, but the level in the defoliated plants remains lower than that of the controls. In the stems the differences in dry weight due to shading have disappeared. It will be recalled that these differences were solely attributable to sugar differences, and in the stem as in the ear sugar level was very low at harvest and the shading difference therefore insignificant. The position with regard to the sheath is similar. Shading of the ear is therefore without effect on the harvest values of dry weight, residual dry weight, or total sugar in all organs except the ear itself, and the number of ears produced is also unaffected. The general effects of defoliation do not differ from those for the 35-day periods. Thus there is a diminishing effect of defoliation on the ear number with later dates of treatment (Table VIII), but no significant difference between the ear numbers in the three groups involving defoliation. The effects on stem and sheath dry weight were confined to the reduction due to the lower ear number, apart from the small difference in sugar level.

The effects of treatment on the dry weights per ear and on the grain weights are shown diagrammatically in Fig. 11. The diagrams have been constructed to show the effects at the four occasions of the treatments E, F, Fs. They are similar in type to the previous interaction diagrams, but here the arrangement is not factorial, and only any interactions between treatment and time of application can be shown. The dry weights per ear in the defoliated plants were greater than those of the controls, but additional removal of the flag-leaf sheath resulted in values below those of the controls. This sheath effect is large at occasion 1 and smaller at the later occasions of treatment. On occasion 2 there is apparently no additional effect when the sheath is removed, but this anomalous result is attributable to an unfortunate chance sample containing an undue proportion of large plants in the Fs group. Measurements of plant heights were made before treatment at occasion 1, and using these values to eliminate, by the method of co-variance the effect of plant size, in so far as it is related to plant height, it was then found that the differences between the treatments F and Fs were significant whether expressed in terms of the weight of 10 plants or as weight per ear. Furthermore, in the grain weights, into which size of the ear does not enter, there is no anomalous value at occasion 2. Shading the ear greatly reduced the dry weight per ear, and the interaction of this treatment with occasion is

TABLE VI

*Dry Weights (gm.), Residual Dry Weights (gm.), and Total Sugars (mg.) of the Organs of 5 Plants of Barley at Harvest after Shading and Defoliation Treatments given on Four Occasions. Treatments as in Table I*

Sowing date: 29 April 1940. Plants harvested: 9, 10, 11, and 12 September. Treatment dates: 28 June, 5, 12, and 19 July.

Occasion with days between treatment and harvest.

	Leaves.				Flag-leaf sheaths.				Stems.				Ears.			
	C	E	C	F	C	E	C	F	A. Dry weights.	Fa	E	EFs	C	F	Fs	EFs
I (73)	2.60	2.29	1.41	0.92	1.46	1.01	8.59	5.61	8.06	6.29	7.71	22.55	13.36	13.27	13.27	7.53
II (66)	3.45	3.08	1.80	1.01	1.93	1.42	10.99	7.27	6.56	8.86	5.96	29.29	15.70	10.93	10.93	6.16
III (59)	2.73	3.08	1.86	0.93	1.42	1.26	8.77	6.35	10.15	6.68	8.76	23.45	16.25	22.34	22.34	11.50
IV (52)	2.36	2.59	1.53	1.25	1.73	0.91	8.41	8.48	6.89	9.67	7.38	23.12	20.91	17.13	21.38	11.10
	2.62	2.59	1.53	1.56	1.82	1.24	8.27	8.22	6.66	8.98	7.23	21.14	15.99	17.13	20.65	12.45
	2.49	2.73	1.41	1.45	1.50	1.24	7.48	7.22	8.59	8.12	8.41	20.08	19.65	19.35	17.46	14.74
	21.99	23.70	12.32	9.63	12.99	12.99	70.03	58.90	62.05	65.98	8.27	21.44	22.27	18.55	17.11	14.64
											61.76	183.50	145.65	138.94	139.52	88.36
I (73)	1.85	1.93	1.43	0.75	1.45	0.75	7.13	5.06	5.70	6.11	5.48	21.73	12.41	13.09	13.09	6.90
II (66)	2.28	2.62	1.68	0.79	1.85	0.79	9.47	5.84	5.45	7.57	5.29	20.66	15.06	10.39	10.39	5.97
III (59)	1.96	1.68	1.49	—	1.30	—	8.00	—	8.26	5.67	7.65	22.32	14.87	20.64	20.64	10.71
IV (52)	1.87	2.43	1.40	1.19	1.64	1.19	7.36	6.31	7.17	8.18	7.21	21.91	15.55	20.10	18.08	10.15
	1.82	2.24	1.38	—	—	—	7.90	7.10	6.13	8.83	6.63	21.81	19.67	16.20	18.04	9.43
	1.68	1.94	—	—	1.72	—	6.36	7.14	5.71	7.74	7.36	19.78	20.11	15.24	15.67	11.40
	1.76	2.04	—	—	—	—	7.13	6.70	7.26	7.27	7.63	19.22	18.97	18.25	13.52	13.52
	1.61	1.70	—	1.35	1.38	—	6.46	7.03	6.72	6.87	6.03	20.81	21.42	17.36	15.80	14.22
	14.83	16.68	—	—	—	—	59.81	—	52.40	58.24	53.28	177.24	138.06	131.36	128.87	82.30
I (73)	36	43	40	12	28	12	257	48	206	264	53	39	46	72	54	45
II (66)	60	55	40	16	30	16	250	36	78	349	57	164	34	51	120	31
III (59)	64	41	41	—	22	27	227	36	39	326	90	451	287	345	205	85
IV (52)	40	48	26	28	23	224	152	52	92	180	84	149	212	107	150	98
	45	62	54	—	59	414	230	127	31	180	37	86	72	22	22	54
	45	54	—	—	—	230	200	53	79	434	86	57	36	63	181	44
	45	37	22	—	20	200	237	165	156	79	73	66	58	118	189	87
	39	38	23	24	—	—	—	—	125	117	95	38	45	56	180	196
	374	378	—	—	—	—	2098	—	896	1910	575	1050	789	835	1166	622

Standard Errors of differences between totals.

Leaves. Sheaths. Ears.

Significant differences between totals 5% level ( $n=8$ ).

Leaves. Sheaths. Ears.

3.1 2.7 8.4 16.1 16.4 67.6



TABLE VII

*Number of Ears, Dry Weight per Ear, Residual Dry Weight of 100 Grains, and Residual Dry Weight of Awns associated with 100 Grains in the Ears of Barley after Defoliation and Shading Treatments. Treatments as in Table I. Treatments given on Four Occasions 28 June, 5, 12, and 19 July. Plants harvested on 9, 10, 11, and 12 September.*

Sowing date: 29 April 1940.

Occasion and days from sowing.		Days between treatment and harvest.	C.	F.	Fs.	E.	EFs.	Occasion totals.
1. Number of ears produced (5 plants).								
I (60)	.	73	25	15	23	22	20	
II (67)	.	67	33	15	16	27	18	214
III (74)	.	61	25	20	22	29	24	231
IV (81)	.	55	26	19	21	28	17	
			25	24	18	33	23	234
			23	23	24	23	22	
			25	22	33	27	28	240
Treatment totals			208	153	169	214	175	

2. Dry weights per ear (gm.).									
I (60)	.	.	73	0.90	0.89	0.58	0.67	0.38	
				0.89	1.05	0.68	0.65	0.34	7.02
II (67)	.	.	67	0.90	1.08	1.01	0.56	0.50	
				0.95	0.82	0.97	0.67	0.46	7.94
III (74)	.	.	61	0.89	1.10	0.82	0.66	0.60	
				0.79	0.88	0.89	0.63	0.54	7.79
IV (81)	.	.	55	0.87	0.85	0.81	0.76	0.67	
				0.86	1.01	0.81	0.63	0.52	7.79
Treatment totals				7.05	7.68	6.57	5.23	4.01	

3. Residual dry weights (gm.) of 100 grains.									
I (60)	.	.	73	4·71	4·61	3·82	3·87	2·60	
				4·59	4·84	4·28	4·08	2·48	39·88
II (67)	.	.	67	4·63	4·53	4·34	4·01	2·92	
				4·78	4·44	4·36	3·88	2·80	40·69
III (74)	.	.	61	5·07	4·61	4·29	3·96	2·95	
				4·78	4·79	4·37	3·76	3·25	41·83
IV (81)	.	.	55	4·72	4·63	4·35	4·23	3·36	
				4·63	4·77	4·40	3·95	3·00	42·04
Treatment totals				37·91	37·22	34·21	31·74	23·36	

4. Residual dry weight of awns (gm.) associated with 100 grains.								
I (60)	.	.	73	0.54	0.95	0.44	0.36	0.46
				0.49	0.67	0.58	0.51	0.37
II (67)	.	.	67	0.73	0.58	0.48	0.37	0.47
				0.55	0.81	0.55	0.49	0.51
III (74)	.	.	61	0.54	0.48	0.48	0.28	0.50
				0.50	0.56	0.53	0.55	0.45
IV (81)	.	.	55	0.63	0.53	0.54	0.38	0.44
				0.49	0.52	0.67	0.45	0.43
Treatment totals				4.47	5.10	4.27	3.39	3.63

Standard Errors of differences between treatment totals	$\left\{ \begin{array}{l} 1. \ 12.00 \\ 2. \ 0.21 \\ 3. \ 0.62 \\ 4. \ 0.34 \end{array} \right.$	Significant differences between treatment totals (5% level. $n = 8$ ).	$\left\{ \begin{array}{l} 1. \ 25.00 \\ 2. \ 0.45 \\ 3. \ 1.31 \\ 4. \ 0.72 \end{array} \right.$
---------------------------------------------------------------	--------------------------------------------------------------------------------------------------	------------------------------------------------------------------------------	--------------------------------------------------------------------------------------------------

also significant, in the expected sense that the effect on the two later occasions is less than that on the two earlier ones. It will be remembered that this interaction was in the opposite sense when only relatively short periods

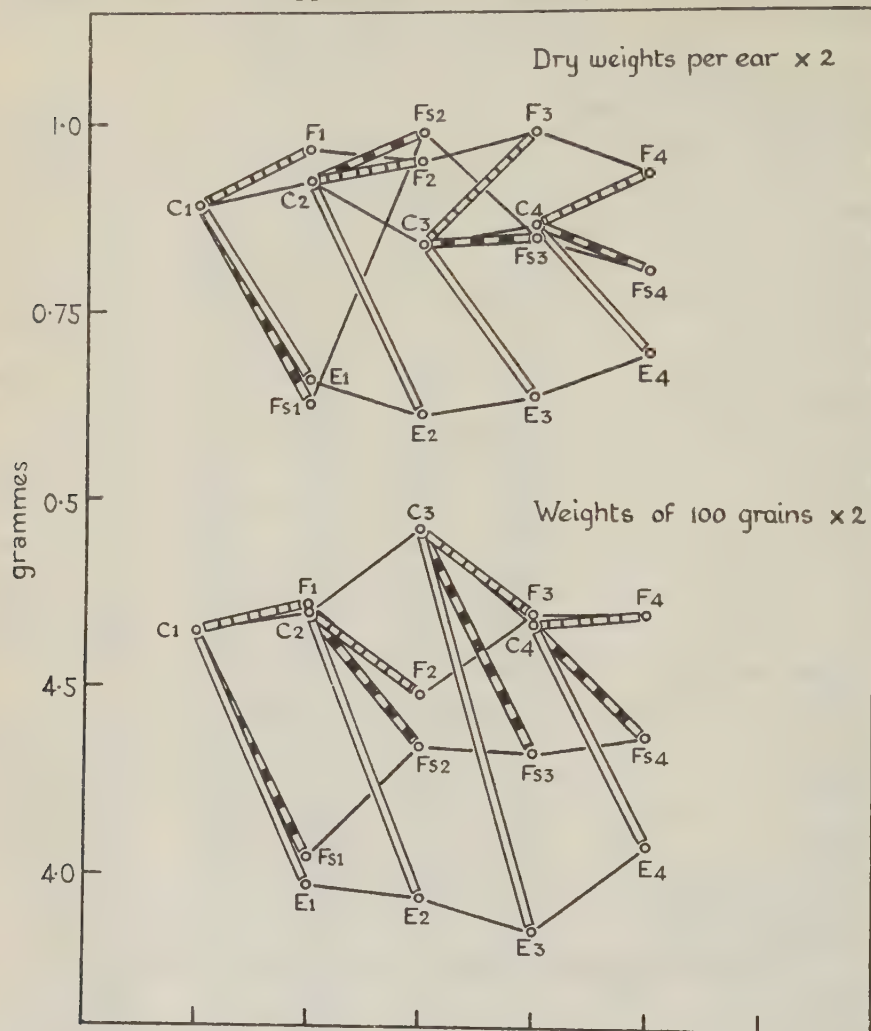


FIG. 11. The effect of treatment on the dry weights per ear and the grain weights at harvest. C, control; F, defoliated; Fs, defoliated and flag-leaf sheath removed; E, ears shaded. Plotted in a similar manner to previous diagrams, although not a factorial arrangement, to show the effect of sheath removal in addition to that of the leaves.

following treatment were considered owing to continued ear emergence during the time between treatment dates.

Grain weight was not reduced by defoliation alone, but the other two treatments lowered it at each occasion, the effect of shading the ear being greater than that of sheath removal. These results confirm the conclusions

reached in the first section, namely that, under the conditions of this experiment, leaves had little or no effect on grain filling, which was dependent on material supplied by the stems and sheaths and by the ears themselves. Since grain size is unaffected by defoliation, while the ears are actually

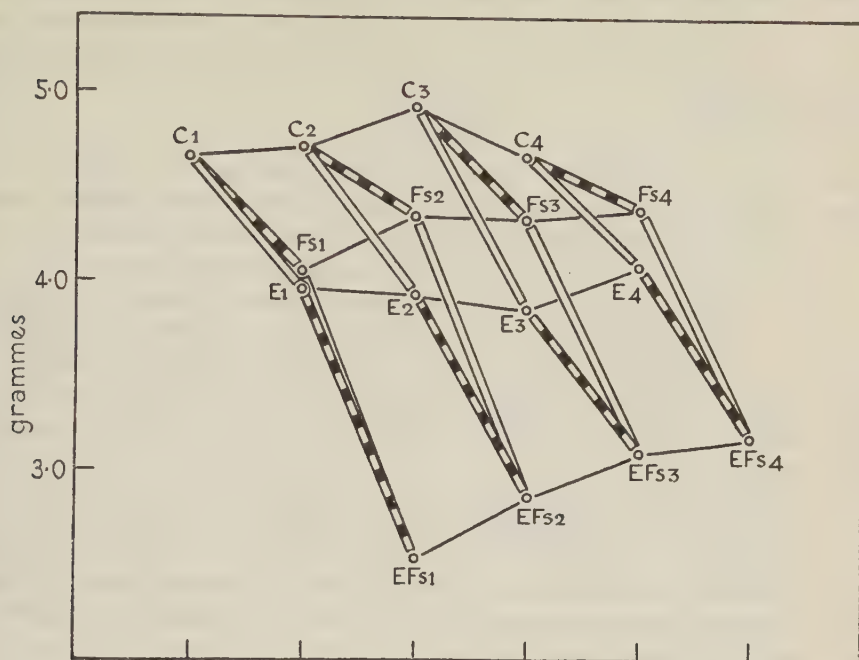


FIG. 12. The effect of treatment on the grain weight at harvest.  
Treatments and arrangement as in Fig. 4.  
Significant interaction  $E \times F_s$ .

larger than those of the control plants, the number of grains per ear must also have been greater in the defoliated samples. This is probably again due to the fact that the small ears on late developing tillers reduce the average values for untreated plants, as compared with those on defoliated plants, where only the large ears produced by the main axis and early tillers are involved.

The grain weights showed no significant interactions between treatments and occasion, so that the interaction between shading and occasion for dry weight per ear must be attributed to an effect on grain number, early shading possibly increasing the number of sterile flowers more than later treatment. Differences arising as a result of variation of the treatment time are, however, small compared with the total treatment effect at harvest; this might be expected, since about 70 per cent. of the total dry weight of the ears is normally accumulated after the time at which the fourth treatment was given.

Grain size showed a highly significant interaction between treatments, which is illustrated by Fig. 12. The effect of ear-shading is here consistently greater with than without defoliation and sheath removal, an interaction

suggested but not proved by the data of the first section. At present no explanation of this interaction can be put forward. The differences between treatment values for the total dry weights and dry weights per ear for the harvest collections are in the expected direction for such an interaction, but these are not significant. The dry weight values for the harvest collections are shown below in Table VIII in the form of  $2 \times 2$  tables, together with the grain weights.

TABLE VIII

*Values of Total Dry Weights of Ears, Dry Weights per Ear, and of the Residual Dry Weights of 100 Grains for Barley at Harvest. The Numbers are the Sums of Eight Values, Two for each of Four Occasions (see Table VI)*

		E. Ears shaded.		Fs. Leaves and flag-leaf sheaths removed.			
		Total dry weight of ears (gm.).		Dry weight per ear (gm.).		Residual dry weight (gm.) of 100 grains.	
		No Fs	Fs	No Fs	Fs	No Fs	Fs
No E	.	183.5	138.9	7.05	6.57	37.9	34.2
E	.	139.5	88.3	5.23	4.01	31.7	23.3
Differences	.	44.0	50.6	1.82	2.56	6.2	10.9

#### ESTIMATES OF THE CONTRIBUTION OF COMPONENT PARTS TO THE DRY WEIGHT OF THE EAR

The percentage reduction in the yield of ears (dry weight), together with other relevant figures are shown below in Table IX. These reductions in

TABLE IX

*Effect of Shading the Ear and of Defoliation Treatments on the Dry Weight of Barley Ears at Harvest (19 Weeks from Sowing). Figures calculated from the Data of Tables VI and VII. Treatments as in Table I.*

Treatment occasion.	Days between treatment and harvest.	Reduction in ear no. (%) due to defoliation.	Total ear growth (%) made before treatment.	Reduction (%) in dry weight due to treatment.				Dry weight increase after treatment as % of total dry weight.			
				F	Fs	E	EFs	F	Fs	E	EFs
1.	73	33	4.3	37	47	30	70	59.1	48.5	66.3	25.6
2.	67	20	12.2	29	5	27	51	58.9	83.2	61.0	37.1
3.	59	17	22.4	8	28	15	51	69.3	49.8	62.9	27.1
4.	52	3	34.2	9	17	25	36	57.2	48.4	41.2	29.8

dry weight constitute the whole effect of ear-shading on the aerial parts of the plant at harvest, but with the treatments including defoliation there are as well proportional reductions in the dry weights of stems and sheaths, because of the lower number of stems developing. The values for treatment Fs on occasion 2 are very aberrant throughout, as is also that for treatment E at occasion 3. The large variability in plant size accounting for these



chance samples has already been discussed (p. 517). The percentage reductions in total dry weight (sheaths, stems, and ears) for the shading and defoliation treatments on the four occasions were respectively

	I.	II.	III.	IV.
E . . . . .	24	22	8	20
F . . . . .	36	27	8	10

so that on the whole defoliation has a greater effect than shading when treatment is given early, while that of shading is the greater when treatment is given late. This difference in the relative effects of the two types of treatment is brought about by the diminishing effect of defoliation as the treatment date advances, the total effect of shading being little influenced by the range of treatment dates considered here.

Estimates of the contribution made by the several organs to the dry weight of the ear may be made from the figures of Table IX. That of the ear itself is directly observed from the results of the shading treatment given on occasion 1, when none of the ears was exposed to full light, all being shaded as they emerged. The value in Table IX is 30 per cent. and since the values for the other three shading times differ little from this figure, it is obvious that the effect is small in the first month during ear emergence, and increases as the ears grow to their full size. The ears therefore contribute substantially to their own growth by assimilation in the seven weeks prior to harvest, the major contribution no doubt being made towards the beginning of this period. This figure of 30 per cent. confirms that already found for barley by Watson and Norman (1939).

The estimation of the contribution of stem (together with sheaths other than that of the flag leaf) from the group of plants receiving the treatment EFs, where only the stem remained exposed to light, is complicated by the effect on ear number of the time of defoliation. Thus on occasion 1, 25.6 per cent. of the total growth (of ears of control plants) was made by the EFs group in the 73 days following treatment, while on occasion 4, 29.8 per cent. was made in the shorter period of 52 days between treatment and harvest, owing to the larger number of stems then available. The stem contribution would therefore be underestimated if the values for the EFs group are accepted without modification. Since defoliation only affects number of ears and not size, estimates of the stem contribution may be made from the data for each of the four treatment occasions by assuming that the increase in yield of the ears is proportional to the number of stems which bear ears, and that the amount assimilated by each stem is proportional to time between treatment and harvest. Thus for occasion 1 the reduction in ear number was 33 per cent., so that 67 per cent. of the total possible number of stems supplied sufficient assimilate to give 25.6 per cent. of the total possible ear dry weight in the 73 days following treatment. Had ear number not been reduced the yield would have been 38 per cent. (i.e.  $25.6 \times \frac{100}{67}$ ). For occasion 2 the reduction in number was 20 per cent. and the time between treatment

and harvest 67 days, the estimated yield calculated from the observed figure of 37 per cent. is 54 per cent. ( $37 \times \frac{100}{80} \times \frac{73}{67}$ ). By similar calculations values of 39 per cent. and 41 per cent. may be obtained for occasions 3 and 4. These estimates agree well, with the exception of that for occasion 2, which seems rather high. It is concluded that the stem contribution is of the order of 40 per cent. Errors in this estimate will arise from the assumption made here that all stems are the same size, and from the further assumption that all stems are assimilating from the time of the first treatment occasion, 60 days from sowing. Both sources of uncertainty will lead to overestimation of the stem contribution, in the former case because the figures are based on the performance of the relatively large stems which survived defoliation and in the latter because in fact all stems were not fully developed 60 days after sowing. The small interaction found between the two types of treatment will lead to errors in the opposite sense, since shading has a slightly greater effect in the presence than in the absence of defoliation. The stem contribution must, however, be greater than 30 per cent. since this value was obtained at the last treatment occasion, so that the margin of error lies between the values 30 and 40 per cent., and the true value is probably nearer 40 per cent.

Similar calculations may be made for the two other defoliation treatments F and Fs, leading to estimates of the contributions of the leaves and flag-leaf sheaths. The results of such calculations for the four occasions give percentage reductions in ear dry weight after defoliation alone (F) of 12, 19, 0, and 17 respectively, which represent the estimated leaf contributions, and for defoliation together with sheath removal of 28, —, 26, and 30, which give by subtraction, estimates of 16, —, 17, and 13 for the flag-leaf sheaths. (Occasion 2 is omitted because of the very aberrant figure recorded for the Fs treatment.) The large sampling errors make these figures somewhat uncertain but an estimate of 15 per cent. may be made for the leaves.

It has already been suggested that the leaves do not contribute to grain filling under the conditions of this experiment, and their contribution should therefore represent the portion of the ear other than the grain, which in these samples was in fact 15 per cent. If now the values for leaves, ears, and stems (15, 30, and 40 per cent.) be subtracted from 100, 15 per cent. remains for the flag-leaf sheath, which is in reasonable agreement with the estimate arrived at above, and derived directly from the Fs and F groups of plants. This figure receives some confirmation from the percentage reduction in grain weight due to sheath removal, which is also found to be 15 per cent. at occasion 1, by comparing the grain weights of treatments F and Fs (Table VII).

## DISCUSSION

It has been shown (Archbold and Mukerjee, 1942) that only about 10 per cent. of the dry material of the barley ear can be supposed to arise from sugar

stored in the stems, if all such sugar is translocated upwards. Since this amount forms such a small proportion of the whole it was concluded that, contrary to the popular view (Belval, 1924), this stored sugar was not essential to normal ear development. If this be the case it becomes pertinent to inquire whether in fact the sugar is lost from the stems by translocation, the only established fact being its disappearance from the stems.

Under conditions of abundant nitrogen supply it has been found (Archbold, 1938a) that barley ears continue to make restricted growth in plants defoliated just before the ear on the main axis emerges, but the normal storage of sugar in the stems is prevented; while Sayre, Morris, and Ritchie (1931) find that defoliation lowers the sugar content of corn stems. These observations together with the well-known fact that removal of ears leads to an immediate rise of sugar in cereal stems (Dehérain and Dupont, 1901; Vilmorin and Levallois, 1913, &c.) make it reasonable to assume that the sugar accumulation results from a temporary excess of assimilate, and that by reducing the flow of such material by defoliation or by shading of the ear an abnormal demand for carbohydrate could be created, and consequently the ability of the plant to utilize the stored sugar tested. Accordingly the defoliation and shading experiments described here were carried out, the treatments being applied at four stages of growth covering the period of rising sugar level in the stems. When the sugar level in the stems was initially high and the ear growing rapidly, no acceleration of the rate of sugar loss, as compared with control plants, occurred in any of the treated plants. The demands of the ear therefore exerted no special influence on the fate of this sugar. When sugar level was initially low, growth of the ears continued, but accumulation of sugar in the stems was restricted. No evidence was therefore forthcoming indicating that the stored sugar was to be regarded as a precursor of starch in the ear. A parallel to this type of effect is that found by Komatsui *et al.* (1933) in sugar-cane. Here defoliated canes continued to grow 'at the expense of sucrose', while 'vigorously growing' canes accumulated sucrose. Possibly the lower sugar level in the defoliated canes may be explained as in the case of barley as a prevention of accumulation rather than as the utilization of sucrose for growth as suggested by the authors. Went (1898), in his studies of seasonal changes in the sugar-cane, states that the sugar was stored in the parenchymatous tissues of the stem, from which it may be inferred that when excess sugar finds its way into the parenchyma it is only removed by respiration or other metabolic processes not involving translocation. In the barley plant therefore stable sugars cannot be regarded as 'available' in the sense that they can be used as substrates for further growth at a site distant from that at which they originally accumulate; they must rather be regarded as surplus to requirements and in the nature of waste products. This view does not take into account the function they may fulfil as a substrate for respiration in the stems. The necessity for the high sugar levels found, solely for supplying respiration needs, is, however, open to doubt since



stems of defoliated plants, where sugar levels are low, can support the growth of normal ears, while losing sugar at a slower rate than untreated controls. Essential respiration requirements can therefore be met when sugar levels obtain which are far lower than those usually found. A discussion of the possible fate of stored sugar in the light of the results already quoted and of those presented here was included in the previous paper (Archbold and Mukerjee, 1942), and no additional data are yet available which warrant further elaboration of this aspect of the problem. It should, however, be emphasized that the arguments are not invalidated by the possible presence of other sources of carbohydrate in the cellulose complexes or the roots. Both sets of experiments showed conclusively that there is no material breakdown of cell constituents which could lead to liberation of sugar; thus the ear depends entirely on primary assimilates for its growth, and stable sugars represent a simple balance of supply over demand.

The effects of defoliation on stems at the different stages of maturity encountered on any one plant at a given time were of two types. Very immature stems were prevented from making further growth, no more leaves were produced and stem elongation and ear development were completely inhibited, an effect similar to that found by Loomis (1935) for maize. More mature stems on the other hand produced full-size ears and defoliation appeared to have no effect apart from reducing the sugar level. The precise stage of tiller development at which the leaves cease to be essential to ear growth in barley is not known, and it is recognized that failure of the immature tillers to continue development after defoliation may not be solely due to restriction of the carbohydrate supply. Leaf removal deprives the tillers of some of the nitrogen, already in short supply, and possibly also of essential hormones; no estimate of the importance of these factors in preventing further growth is possible. With the partially N-deficient plants of the present experiments there appeared to be no intermediate stage when ears continued to grow but failed to attain full size, whereas in an earlier experiment (Archbold, 1938*a*), in which four defoliated tillers only were allowed to remain on plants receiving abundant N, the ears continued to grow but the maximum size reached fell short of that of control plants. The size of the barley ear is reduced by shortage of nitrogen and this dependence of the size of the ears on N supply may have some bearing on the difference of behaviour in the two sets of defoliated plants. In the N-deficient group the production of ears equal in size to those of undefoliated controls implies that sufficient assimilate can be produced by organs other than the leaves to maintain the limited growth permitted by the available nitrogen and at the same time to allow a small amount of sugar to accumulate in the stems. Where nitrogen was abundant the control plants produced large ears, and in the absence of leaves sufficient assimilate was not apparently forthcoming to supply the additional carbohydrate necessary to maintain normal ear growth under those conditions; so in this group of plants sugar was virtually absent



from the stems and ears were undersized when all available material was utilized.

The function of the leaves in ear growth thus appears to be twofold, first they are essential to the formation of the ears, and second, where conditions favour maximum growth, they also supply part of the material for the filling of the grain. On the basis of the experiment with abundant nitrogen this constitutes up to 30 per cent. of the total. If growth is limited by factors other than carbohydrate supply, then the contribution of the leaves may fall to 15 per cent., as in the present case, and not include any part of the grain filling. In these circumstances there occurs the high sugar level in the stems characteristic of nitrogen deficiency; and it may be suggested that assimilation by the leaves rather than by stems themselves is the source of stored sugar, and that material assimilated by the stem is more accessible to the ear than that coming from the leaves. The effects of defoliation become less the later in the growth cycle the leaves are removed, due no doubt to the increasing proportion of senescent leaves. The time during which leaves are able to supply assimilate will also vary with growth conditions. Leaves die off more rapidly when nitrogen is in short supply, and the result of the shorter life as compared with full nitrogen conditions will show itself, not as an effect on the ear which can be fully maintained in the absence of leaves, but on the sugar level in the stems, which instead of being maintained near the maximum value for some time, as is the case where nitrogen is abundant, falls rather rapidly from the maximum (see Archbold and Mukerjee, 1942). To determine whether sugar level in the stems is actually dependent only on the activities of the leaves, in relation to the ear growth, would require experiments in which the stems are shaded and not the leaves, but it is certain that this accumulation of sugar is restricted or even prevented by defoliation, and that the distribution of leaf assimilate between stem and ear depends on conditions governing ear growth, other than the carbohydrate supply. Once stabilized in the stem the sugar is not again available for the ear; in fact, when sugar normally accumulates, the ear is growing at nearly its maximum rate, while later not only has the rate of growth fallen, but the ear itself has developed its full assimilating power and the demand for carbohydrate supplies from elsewhere might be presumed to be lessening. It may also be noted that no reduction in sugar level in the leaves followed shading of the ears, and indeed leaf metabolism appeared quite unaffected by this treatment and therefore independent of the carbohydrate requirements of the ear.

When the flag-leaf sheath is removed in addition to the leaves, grain weight is reduced, the effect being large when treatment is given early and small when given late. Sheaths thus contribute to starch storage until they become senescent. In plants with shaded ears the sugar level in the sheaths was unaffected, but in defoliated plants it was lowered, so that as in the stems the sugar stored in the sheath was not available to the ear when a demand was

artificially created; again when leaves were removed as a source of supply the sugar accumulation diminished.

As a result of shading the ear the grain weight is much reduced, but at the same time the sugar level in the stem is only slightly lowered. The ear can thus only draw on a limited amount of the assimilated material which appears as sugar in the stems of untreated plants, in order to keep up its supply of substrate for starch formation. This contrasts with the situation arising after defoliation where sugar level is low but the ears store the normal amount of starch. The issue at once arises as to what parts of the stems are active in supplying the ears. Few data in regard to regional sources of supply are available for barley, but it is known that sugar accumulates successively in the stem internodes from bottom to top, as each makes its extension growth, and may reach a maximum and begin to decline in the lower internodes while still rising in the upper. Went (1898) found that the lower internodes of the sugar-cane do not continue accumulating sugar when fully grown, but each accumulates it rapidly as it matures. Suzuki and Ishii (1935) found that in any single internode of sugar-cane the sugar concentration varied from top to bottom and from without to within, while de Cugnac (1931) made a similar observation for grasses; so there is a periodic variation in sugar content along the stems of these plants. Das (1936) considers accumulation of sucrose in cane a process individual to each internode, which ceases when the attached leaf dies or falls off; he finds that when flowering occurs there is no general withdrawal of sugar from the stem, only adjacent internodes being affected. Finally Boonstra (1929) states that only the top two internodes of the wheat stem contribute to the growth of the ear. The evidence available clearly suggests that each internode develops independently of the rest, and there is no ready interchange of sugar between internodes, a point of view already reached with regard to barley by the study of seasonal changes of carbohydrates (Archbold and Mukerjee, 1942). Such a situation would be compatible with the sugar changes resulting from shading the ear, which treatment may be supposed to divert to the ear assimilate from those internodes which are still accumulating sugar at the time of shading. The lower level of sugar in shaded plants will then be due solely to prevention of maximum sugar accumulation at the top of the plant while lower internodes are unaffected.

In the present experiments stems and sheaths other than that of the flag leaf supplied 40 per cent. of the total material to the ear, and therefore play a most important role in ear development. How much is due to the sheaths and how much to the stems is not known; but it is likely, as explained above, that the large top internode makes a vital contribution, especially as it remains green very late in the growth cycle and long after the sheaths of the lower internodes are dead. The ears contributed 30 per cent. and the flag-leaf sheath 15 per cent., making a total of 85 per cent. by organs other than the leaves. The essential function of the leaves, as has already been pointed out,

is thus not grain filling, but lies in the part they play in ear formation at the outset. Under some conditions of growth they may contribute to grain filling, but they do not appear to be necessary; the primary sources for this purpose being the stems and the ears themselves.

If it is accepted that stored sugar in barley and other monocotyledons such as wheat and sugar-cane is not a reserve available for subsequent growth processes, the reserve function of accumulations of sugar in other plant types may perhaps be questioned. Colin (1916, 1917) in his well-known researches on the beetroot showed that the sugar in the root is not necessary for the production of the flowering stem, since if the crown alone is planted a flowering stem is produced. Furthermore, in the winter following the first year's growth only very little sugar was lost from roots left in the ground, and during the second year's growth sugar in the root at first increased rather than decreased. Colin also calculated the amount of sucrose which might be exported from leaves at night on the basis of the fall in sugar content, and found the amount insufficient to account for the root sugar as well as for respiration requirements; he thus concluded that translocation also proceeded by day. His investigations on inulin (Colin, 1919) led to the same main result, namely that the inulin stored in the artichoke tuber was out of all proportion to the needs of the flowering stem. The same general interpretation is clearly possible here as in the case of the monocotyledons. Sugar is produced in excess of growth requirements and becomes isolated in parenchymatous tissue in the roots and tubers, where it is lost by respiration or other metabolic process, but not by translocation, the flowering stem exercising no particular control over its fate. To these examples of so-called storage sugar in monocotyledonous stems and in roots and tubers may be added that of sugar in the tissue of fleshy fruits, which for obvious reasons is not regarded as a storage reserve available for later use. If the views put forward here are correct, then all these types of storage tissue accumulate sugar solely as a result of the supply being in excess of a demand controlled by factors other than carbohydrate metabolism. Such excess sugar finds its way into the parenchyma from which it is lost at varying rates by conversion to non-reducing substances, one of which is carbon dioxide.

#### SUMMARY

Ear shading and defoliation treatments were applied to barley plants grown under conditions of partial N deficiency. Treatment was given on four occasions at weekly intervals. The first (60 days after sowing) was at the time of emergence of the ear on the main axis, when sugar level in the stems was low, and the fourth (81 days after sowing) when most of the ears had emerged and sugar level in the stems was at its maximum.

Dry weight, residual dry weight, and total sugar were estimated in samples taken 5, 17, and 35 days after each treatment occasion, and again at the



normal harvest date (19 weeks after sowing), with the object of examining the effect of treatment on the sugar metabolism and of estimating the contributions by direct assimilation to the dry weight of the ear of leaves, sheaths, stems, and ears.

The treatments given were (1) defoliation, (2) defoliation accompanied by flag-leaf sheath removal, (3) shading the ear, and (4) 2 and 3 combined.

Treatment effects on stems and sheaths were confined to alterations in the sugar level and there was no breakdown of residual dry weight to supply assimilation deficiencies. Sugar levels are therefore the simple resultant of production and utilization of assimilate and are not complicated by production of sugar from insoluble reserves. Plants treated when sugar levels were low failed to store sugar in the stems in the normal manner, but plants treated when sugar levels were high lost sugar at the normal rate. The alteration in sugar level is thus brought about by restriction of sugar accumulation and not by accelerated utilization, although the conditions supposedly create a demand for carbohydrate in the ear. It is concluded that sugar stored in the stems and sheaths is in the nature of a surplus and is not available for subsequent conversion to starch in the ear, but is lost from the stem by respiration and other metabolic processes not involving translocation.

Defoliation alone reduced the number of ears reaching maturity but not their size, or the grain size. Under the conditions of this experiment therefore leaves made no contribution to grain filling, but were of paramount importance in ear formation. Early removal of the flag-leaf sheath reduced grain size, but late removal had no effect. The level of sugar in the stem was greatly reduced by defoliation and it is suggested that assimilation by the leaves is the source of stem sugar, rather than the activity of the stems themselves.

Shading the ears reduced the size of the ear, but did not affect the number produced. Stem sugars suffered only a small reduction, so that the shaded ears were only able to utilize a small part of the material which would otherwise have appeared as sugar in the stems.

The carbohydrates of the ear are thus entirely supplied by directly assimilated material, translocated immediately from the various organs. The extent to which each contributes, calculated from observations on samples collected 19 weeks after sowing, was: leaves 15 per cent., flag-leaf sheath 15 per cent., stems and other sheaths 40 per cent., and ears 30 per cent. There is some evidence that these proportions may be affected by growth conditions. Limitation of ear size by nitrogen shortage is thought to account for the small leaf contribution, the assimilate appearing instead as sugar, and leading to the high levels characteristic of nitrogen deficiency. If this be so, then the stems afford more accessible supplies of assimilate than the leaves.

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# Studies in the Comparative Morphology of the Algae

## II. The Algal Life-Cycle

BY

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With one Figure in the Text

BY contrast with the stereotyped phase-alternation characteristic of archegoniate plants, the Algae exhibit a considerable diversity in their life-cycles. In view of the great increase in knowledge in this respect during the present century, the time seems ripe for a general survey of the algal life-cycle with the object of sorting out the essential facts and of stating the conclusions that can be deduced from them. It is in the main among Chlorophyceae, Phaeophyceae, and Rhodophyceae that sexual fusion commonly occurs at some place in the life-cycle, to be followed at some other stage by a compensating reduction. As a general rule, but not invariably, this cytological alternation is concomitant with an alternation of phases which are distinguished either only by the reproductive organs they bear or by other morphological features as well. When the two alternating phases differ only in the number of chromosomes ( $2n$  and  $n$ ) and in the type of reproduction exhibited, I speak of the alternation as *isomorphic*, whilst, when the two phases differ also in vegetative characters, the alternation is designated *heteromorphic* (Fritsch, 1935, p. 52). These terms are more satisfactory than 'homologous' and 'antithetic', which convey implications that are not necessarily applicable to all examples; moreover, the word 'antithetic' is applied by many continental writers to all instances in which there is a definite cytological alternation, whether the phases involved are isomorphic or heteromorphic.

### HAPLOID AND DIPLOID FORMS

Wherever sexuality occurs among unicellular and colonial Algae, reduction for the most part ensues during the first two divisions of the fusion nucleus so that the zygote constitutes the only diploid stage in the life-cycle. This probably resembles the primary condition that followed immediately upon the appearance of sexuality and is illustrative of a stage prior to the establishment of a definite phase-alternation. Haploid organisms of this type are widespread in the Chlorophyceae (Volvocales, Hydrodictyaceae, Ulotrichales, Chaetophorales, Oedogoniales, Conjugales), and it is noteworthy that, so far

as present evidence goes, all oogamous members of this class exhibit this kind of life-cycle. The latter is, moreover, encountered in all classes in which the majority of the members are flagellate or unicellular (Xanthophyceae, Chrysophyceae), in so far as sexuality occurs in them at all.

In all such instances the haploid individual propagates abundantly, and often for long periods, solely by means of motile or other kinds of asexual reproductive cells, but these lead merely to reproduction of the same phase; they are not distinguished by any differences in chromosome-number and are not fundamental to the actual life-cycle which involves sexual reproduction, zygote-formation, and subsequent reduction. These methods of reproduction of the haploid phase can therefore be described as *accessory*. During the germination of the zygote there normally ensue only the two meiotic divisions which result in the production of 4 individuals (Mesotaeniaceae) or 4 reproductive cells (Ulothrix, Oedogonium) that give rise to the new haploid phase, although variety that cannot be considered here is introduced by suppression of some of the products so that commonly only one survives (Eudorina, Spirogyra). Where the germinating zygote produces motile reproductive cells, these are of the same type as the accessory zoospores of the haploid plant (Ulothrix, Oedogonium). Alone in Coleochaete, remarkable also in other respects among the oogamous Chlorophyceae, does germination of the zygote result in the formation of a larger number of haploid cells, a condition paralleled among the Bangiales.

A direct derivation of diploid from haploid types is probable and not difficult to visualize. The long-continued motility of the zygote in diverse Chlamydomonadaceae (Korschikoff, 1926, p. 485; Pascher, 1927, pp. 68, 294; cf. also Fritsch, 1935, p. 119) entails a prolongation of the diploid phase which, if it were accompanied by temporary suppression of the tendency to form a resting stage, might lead to the evolution of a diploid type. Some Carterias may possibly have arisen in this way from species of Chlamydomonas, although cytological data in support of such a view are at present lacking. Mainx (1931, p. 516) describes instances in which the zygotes of Oedogonium produce but a single large zoospore which grows into a thread of twice the usual width, differing also in other respects from the normal; these plants are presumably diploid. If either of these examples are valid instances for the origin of diploid types, there is no reason to suppose that they would necessarily result in any prolonged deviation from the normal life-cycle, the diploid individual subsequently behaving as a haplont and undergoing reduction at the time of zygote-germination.

On the other hand, prolongation of the diploid phase as above, with the elimination of all accessory reproduction, might result in the postponement of meiosis to the time of gametogenesis. The diploid zygote would then have become the vegetative individual, while the haploid phase was represented solely by the gametes. Such a change would admit of indefinite spinning out of the diploid phase and, if unaccompanied by the formation of septa, would



result in forms such as are represented among Siphonales. There is evidence that several of these are diploid, as was first established for *Codium* by Williams (1925) and has since been recorded for *Acetabularia* (Schussnig, 1929, p. 273), *Bryopsis* (Schussnig, 1932; Zinnecker, 1935), and *Valonia* (Schechner-Fries, 1934; Schussnig, 1938). *Valonia* differs from the others in the fact that there is also copious accessory reproduction by diploid biflagellated swimmers resembling the gametes. The same condition is found in *Cladophora glomerata* (Schussnig, 1928; List, 1930; Schussnig, 1938, p. 56) which propagates by diploid biflagellate zoospores all the year round, but only produces gametes after meiosis in spring. *Valonia* is not the only member of Siphonales with a life-cycle different from that hitherto assumed to be the rule in this order, and its further consideration is deferred to p. 536.

A considerable number of Siphonales would, however, appear to be true diplonts propagating solely by gametes. They contrast essentially with the haploid Green Algae only in an inversion of the importance of the two cytological phases, since in them it is the diploid that is dominant, while the haploid is restricted to the sexual cells. This is, of course, also true of *Valonia* and *Cladophora glomerata*. Such an inversion could be accomplished without difficulty in a unicellular form, and there is therefore, apart from other considerations (Fritsch, 1935, p. 369), reason to suspect a direct derivation of Siphonales from unicellular types. There is some evidence for the existence of diploid types among Chlorococcales. This has been definitely asserted for *Chlorochytrium* (Kurssanow and Schemakhanowa, 1927) and is possibly also true of *Chlorococcum* and certain allied forms (Korschikoff, 1926, p. 485), although all are characterized by frequent reproduction by swimmers that exhibit no fusion. Such accessory reproduction is to be expected in relatively primitive unicellular types. Diploid Chlorococcales might easily arise from motile unicellular haplonts in the way postulated above.

Diploid organisms undergoing reduction during gametogenesis are also frequent among pennate Diatoms. The obscure relations between them and the centric forms, as well as with other classes of Protophyta, renders speculation as to the origin of the diploid condition in them unprofitable. Certain diploid forms among Ectocarpales are considered below (p. 546), while the relationships of the diploid Fucales will be discussed in the third article of this series.

#### ISOMORPHIC ALTERNATION

The existence of two morphologically identical phases, the haploid one reproducing sexually, the diploid one asexually, has now been established for a considerable number of Algae, viz. the Ulvaceae, diverse *Cladophora*-ceae, and certain Siphonales among Chlorophyceae; the Ectocarpaceae, Dictyotales, and in all probability Tilopteridales and Sphacelariales among Phaeophyceae; and in the diplobiontic Florideae where the phenomenon is complicated by the occurrence of a second dissimilar diploid phase.

(a) *Chlorophyceae*

The two families of Chlorophyceae first mentioned are related to the haploid Ulotrichaceae and are both distinguished by somatic specialization. The Ulvaceae are plainly derived from forms resembling the present-day Ulothrix, both *Ulva* and *Enteromorpha* passing through a simple filamentous stage of more or less prolonged duration before septation in other planes sets in. The Cladophoraceae are distinguished by their multinucleate cells and elaborate chloroplast, the frequent extensive branching with formation of reproductive cells only in the ultimate laterals, and the occurrence of more or less clearly defined apical growth. The existence of vegetatively simple haploid types and of others, with more advanced somatic organization and isomorphic alternation, among families of Chlorophyceae that afford evidence of affinity to one another justifies the hypothesis that the isomorphic forms may have originated directly from haploid types (see, however, below). In the majority of the latter there is normally a long succession of plants reproducing only by asexual means, prior to the appearance of sexual individuals. In other words, there are, even here, asexual and sexual generations, although both are haploid. The postponement of meiosis from the zygote to the end of the asexual phase is generally supposed to have taken place as a sudden mutation, and with it the resting period of the zygote, usual in haploid types, was abandoned. The change involved is somewhat analogous to that probably occurring in the evolution of diploid types, a diploid vegetative individual being intercalated between the process of sexual fusion and the occurrence of meiosis, although the products of the latter are haploid zoospores giving rise to individuals in which gametogenesis is deferred until the end of the vegetative phase.

An isomorphic alternation has also recently been reported in *Anadyomene* and *Microdictyon* among Valoniaceae (Iyengar and Ramanathan, 1940 and 1941); vegetatively identical individuals here reproduce respectively by quadriflagellate zoospores and biflagellate gametes. In *Microdictyon* meiosis during zoospore-formation is recorded, although the data provided are somewhat scanty. Since it appears definitely established that *Valonia* itself undergoes reduction during gametogenesis as in *Codium*, the observations on *Microdictyon* and *Anadyomene* raise the problem of the relation between purely diploid types and those showing an isomorphic alternation. A similar problem exists among Cladophoraceae (cf. above).

Both in *Valonia* and in *Cladophora glomerata* the diploid individuals reproduce by asexual, as well as by sexual means, although so far as the evidence goes gamete-production takes place in individuals that do not produce diploid asexual swimmers. In other words, in both instances there are, as in haploid types, sexual and asexual phases, although both are here diploid. The occurrence of meiosis in the asexual rather than in the sexual individual would give an isomorphic alternation like that recorded for diverse Cladophoraceae and for *Anadyomene* and *Microdictyon*. In considering the possible deriva-

tion of isomorphic from diploid types, themselves originating from an elaboration of the zygote of a haploid ancestor, it must be realized that reduction might just as well ensue during the production of asexual as of sexual swarmer. If the latter occurred (*Valonia*, *Cladophora glomerata*) the form in question would remain a diplont; if the former an isomorphic alternation would result. It is therefore possible that both the purely diploid and the isomorphic types met with in Siphonales may have evolved along divergent lines from a common source. It is, moreover, not out of the question that isomorphic forms in other groups also might have originated from diploid types, arising perhaps in the way suggested by Mainx's observations on *Oedogonium*.

On the other hand, the diploid types found in Siphonales and Cladophorales might be derived from forms with an isomorphic life-cycle, a change for which there is some analogy in Ectocarpales and certain other Phaeophyceae (see p. 540). Such a derivation would involve the assumption of sexuality on the part of some of the swarmer of the diploid phase, while others arising without reduction subserve accessory reproduction; elimination of the latter would give the condition found in *Codium*, *Bryopsis*, &c. In this connexion it is noteworthy that Higgins (1931), in the apparently isomorphic *Cladophora flavescens*, observed occasional fusions between the zoospores. The relatively low degree of specialization of *Valonia* as compared with *Anadyomene* and *Microdictyon* renders this hypothesis improbable, but it is scarcely profitable to discuss the alternatives further until more data are forthcoming. It is to be hoped that tropical workers, favourably situated for the investigation of these forms, will explore this rich field, and Iyengar is to be congratulated on the progress he has already made in this direction. Attention may be drawn to the fact that in *Valonia* and *Cladophora glomerata* both swarmer-types are dikontan, whereas in *Anadyomene* and *Microdictyon* the asexual are tetra-, the sexual dikontan. In this respect the two last-named genera resemble the Cladophorales and, since segregative division has not yet been demonstrated in them, the possibility that they are actually members of Cladophorales must be visualized.

Yet another type of life-cycle has been recorded among Siphonales for *Derbesia* (Kornmann, 1938), the zoospores of which are stated to give rise to sexual individuals indistinguishable from the alga *Halicystis*, with which *Derbesia* is commonly associated in nature. These statements require verification, since Hollenberg's (1935) profound study of *Halicystis* affords no evidence in support of them. Should they be confirmed, *Derbesia* would show a heteromorphic alternation, analogous to that suspected in *Urospora*, (cf. Fritsch, 1935, p. 241) and, as there, no doubt derived from an isomorphic one, *Cutleria* (cf. also Fritsch, 1942, p. 409).

The high organization attained by the Chaetophorales justifies the expectation that a phase-alternation may have arisen in some of them. *Coleochaete* is, however, haploid, as are also certain species of *Stigeoclonium* (Godward,



1942) and, while there are some indications of a possible alternation among Trentepohlias and a few other Chaetophorales (Fritsch, 1935, p. 290), the data are at present too fragmentary to admit of any satisfactory conclusion. In *Stigeoclonium subspinosum*, according to Juller (1937), the zygote gives rise to a few-celled diploid plant, the swarmers of which regenerate the haploid stage. The positive evidence of a phase-alternation afforded by this species calls for the investigation of other Chaetophorales from this point of view. The diminutive size of the diploid phase is possibly but a cultural effect. Since zoospores and gametes develop on distinct plants in *Fritschiella* (Singh, 1941, p. 171) and the zygote germinates directly, this curious terrestrial type probably shows an isomorphic alternation.

### (b) *Phaeophyceae*

Before discussing alternation in this class it is advisable to clarify the picture by the statement of certain facts, since essential features are easily lost sight of amid the mass of accumulated data. Of the two types of reproductive organs (uni- and plurilocular sporangia) met with in the less specialized Brown Algae, the unilocular sporangia appear almost without exception to constitute asexual organs, their zoospores germinating without fusion to produce a plant which is potentially a gametophyte. That the first two nuclear divisions in the unilocular sporangium bring about reduction has now been demonstrated in so many members of the class that, unless there is definite proof to the contrary, the plants bearing such sporangia must be regarded as diploid and sporophytic (cf. Svedelius, 1928, p. 301; Kylin, 1933, p. 75) and the swarmers produced in them as haploid. There is no good evidence that reduction can occur at any other stage in the life-cycle, although Knight (1923, p. 355) states that on certain diploid plants of *Pylaiella* the last divisions in the plurilocular sporangia showed the haploid number of chromosomes.

Apart from this, there is nothing to indicate that the swarmers liberated from plurilocular sporangia ever differ in chromosome-number from the parent plant. Where such sporangia occur on individuals also bearing the unilocular type, their swarmers must be diploid, as has indeed in various instances been proved; they constitute merely a means of accessory propagation of the sporophyte. When plurilocular sporangia occur on what is known to be a haploid thallus, they are invariably *unaccompanied* by unilocular sporangia and have in several instances been shown to produce gametes, the resulting zygote giving rise to the new diploid phase. The frequent absence of observed syngamy cannot be taken as definite proof that the swarmers in question lack sexuality, since the artificial environment of a laboratory is only too likely to create conditions inimical to the occurrence of sexual fusion (cf. p. 544); this is, moreover, dependent on outside factors (Kniep, 1928, pp. 146, 154; Hartmann, 1934, p. 135).



The customary close resemblance between the accessory plurilocular sporangia on diploid individuals and those which function as gametangia on the haploid individuals is one of the reasons why the life-cycle of Ectocarpales so long remained obscure. The investigations of the last two decades have, however, clearly demonstrated that the complete life-cycle in this order involves an alternation between (a) a diploid individual bearing asexual (unilocular) sporangia (often accompanied by accessory plurilocular sporangia), and (b) a haploid individual bearing plurilocular sporangia which function as gametangia. There is every reason to believe that the two phases are morphologically identical in many of the simple filamentous Ectocarpaceae, and this would seem to represent the primary condition among present-day Phaeophyceae. Although an original derivation from haploid types is to be suspected, none such have so far been found in this class.

The oft-investigated life-cycle of *Ectocarpus siliculosus* is particularly instructive in relation to alternation in Phaeophyceae and may be discussed in some detail. Berthold (1881) first showed that the plants found at Naples in spring liberate gametes from the plurilocular sporangia, the only kind of reproductive organs present, and this has subsequently been corroborated by several investigators, while Knight (1929, p. 318) established that these plants are haploid. Berthold failed clearly to settle the fate of the zygote, although some evidence was produced (1881, p. 412) for its development into a small plant forming both uni- and plurilocular sporangia, and more recently this has been confirmed (Föyn, 1934, p. 4; Schussnig and Kothbauer, 1934, p. 91). The sporophyte is a winter form, appreciably smaller than the gametophyte and bearing, apart from unilocular, accessory plurilocular sporangia.

A similar alternation has been established by Föyn (1934, p. 6) for *E. siliculosus* on the coast of Norway and by Papenfuss (1935a) at Woods Hole in North America; in both regions there are haploid plants with plurilocular sporangia only, and diploid plants with pluri- and unilocular sporangia, either on the same or on distinct individuals. The diploid plants at Woods Hole, however, are more robust than the haploid ones, having bigger cells and considerably larger plurilocular sporangia. It would thus seem that in northern waters, by contrast to the state of affairs obtaining in the Mediterranean, the diploid plant of *E. siliculosus* is the more vigorous one. This is in harmony with the fact that Knight (1929, p. 322) in the Isle of Man found only diploid plants (cf. also Kylin, 1933, p. 16; Levring, 1940, p. 31). The occurrence of reduction in the unilocular sporangium of this species has been demonstrated by diverse workers (Knight, 1929, p. 314; Papenfuss, 1935, p. 435; Schussnig and Kothbauer, 1934, p. 88).

The varying vigour of sporophyte or gametophyte in different regions implies a capacity towards differentiation between the two phases which is significant in relation to the origin of heteromorphic alternation in Phaeophyceae. The apparent absence of the gametophyte in certain localities is,

moreover, noteworthy. At the same time there is evidence that yet another modification of the life-cycle may obtain. Knight (1929, p. 316) in her British material describes fusion of the swarmers from the unilocular sporangia, also reported (p. 321) for certain other species of *Ectocarpus* and (p. 322) for *Pylaiella*, although the data are perhaps not absolutely convincing (cf. also Kylin, 1933, p. 19; Papenfuss, 1935, p. 423). The same state of affairs has, however, been recorded in *E. siliculosus* also in the Adriatic (Schussnig and Kothbauer, 1934, p. 85), as well as in several of the more advanced Ectocarpales (Knight, 1929, p. 317; 1931, p. 23; Knight and Parke, 1931, p. 109; Abe, 1935a; Hygen, 1934, p. 257), in *Desmarestia* (Abe, 1938), and in certain Sphacelariales (Clint, 1927, p. 17; Knight, 1929, p. 317), and these data strengthen the view that such behaviour on the part of these normally asexual swarmers is possible. I am inclined, however, to regard this condition as exceptional. We have here apparently a complete elimination of the gametophyte, the asexual cells that normally produce it themselves behaving straight away as gametes.

All who have studied sexual reproduction in *E. siliculosus* report that a certain percentage of the gametes fail to fuse. Such gametes may develop apogamously, with the production of further plants bearing plurilocular sporangia only (Berthold, 1881, p. 412), and in this way accessory reproduction of the haploid phase is accomplished. In the normal life-cycle of the species under discussion, therefore, either phase can propagate indefinitely by non-sexual means, the diploid one with the help of the swarmers from its plurilocular sporangia, the haploid one with the help of apogamous gametes. This plasticity in methods of reproduction appears to be characteristic of the more primitive members of Phaeophyceae.

The successive generations, which may thus for long periods perpetuate the diploid or haploid phases, as the case may be, as a general rule in *Ectocarpus* probably display no essential differences from the normal adult thallus, but this is not always so. In *E. tomentosus* Sauvageau (1928) describes such stages as simple ectocarpoid growths, showing nothing of the intertwining of the threads characteristic of the adult plant; they are also distinguished by the hairs which they bear.

The probability that a life-cycle, comparable to that of *E. siliculosus*, occurs also in other species of the genus is supported by the data relating to *E. virescens* (Sauvageau, 1933, p. 67; Boergesen, 1939, p. 75) and *E. Hincksiae* (Sauvageau, 1933, p. 23). The evidence also points to a similar alternation obtaining in other Ectocarpaceae. Thus, the individuals of the true *Pylaiella littoralis* on the coasts of Scandinavia for the most part bear either uni- or plurilocular sporangia (Kylin, 1937, p. 3; Levring, 1937, p. 44); the former, which are diploid (Knight, 1923, p. 350), differ from the haploid in their larger size and in certain other respects (Kylin, 1933, p. 10). In Britain plurilocular sporangia occur also on the diploid individuals, whilst haploid plants are very rare (Knight, 1929, p. 322). Fusion of the gametes produced

by the haploid phase has been observed (Knight, 1923, p. 354; Kylin, 1933, p. 14), although apogamy is also reported. Damman's (1930, p. 8) record of unilocular sporangia on the haploid individuals of *Pylaiella*, the only instance of such occurrence so far reported is believed by Kylin (1937, p. 6) to be due to confusion with the lithophytic *P. rupicola* (cf. p. 547). The existence of isomorphic alternation is also probable in *Sorocarpus* (Takamatsu, 1936; Kylin, 1937, p. 20) and *Geminocarpus* (Skottsberg, 1921, p. 9), although sexuality on the part of the swarmer from the plurilocular sporangia has only been demonstrated in the first (Abe, 1935).

While, therefore, a considerable number of the simple filamentous Ectocarpaceae appear to possess an isomorphic life-cycle, there is little evidence of its occurrence among the more specialized Ectocarpales; in other words, elaboration of both phases is a relatively rare phenomenon in this order. There is considerable probability, however, that both *Nemoderma* (Kuckuck, 1912) and the *Lithoderma fatiscens* of Kuckuck (1912a; Kylin, 1918, p. 41) show isomorphic alternation, although no cytological data are at present available for either genus. It is also possible that other Myrionemataceae (cf. *Ralfsia*; Kylin, 1934, p. 17) as well as *Strepsithalia* (Sauvageau, 1896, p. 59) possess an alternation of this type. Another probable instance is furnished by the Pacific *Heterochordaria abietina*, in which uni- and plurilocular sporangia occur on distinct individuals (Setchell and Gardner, 1925, p. 550; Abe, 1935a) and the swarmer of the plurilocular sporangia have been shown to behave as gametes (Abe, 1935); reduction in the unilocular sporangium has also been demonstrated (Abe, 1936). So far there is no evidence of the occurrence of isomorphic alternation among the polystichous Ectocarpales.

On the other hand, it is just among the other parenchymatous Phaeophyceae that the most striking instances of isomorphic alternation occur. The Dictyotales have long been classical examples of such alternation and, although precise data are as yet very scanty, it is probable that the Sphacelariales have a life-cycle of this type. It is noteworthy that both orders are distinguished by their well marked apical growth. The Cutleriales, however, show that the retention of isomorphic alternation was not restricted to such forms alone (Fritsch, 1942, p. 406). The cytological alternation of Dictyotales has been proved for *Dictyota* (Williams, 1904), *Padina* (Georgevitch, 1918 and 1918a; Carter, 1927), and *Zonaria* (Haupt, 1932), and has also been established experimentally for the first two (Hoyt, 1910; Wolfe, 1918). The frequent preponderance or sole presence of asexual plants in certain regions (cf. Reinke, 1878, p. 33; Sauvageau, 1905, p. 68; Williams, 1904, p. 156; Funk, 1927, p. 364; Robinson, 1932, p. 114; Haupt, 1932, p. 244) is not yet fully explained, but the repeatedly recorded germination of the entire contents of a tetrasporangium (Reinke, 1878, pp. 9, 24; Robinson, 1932, p. 117)—presumably without the occurrence of reduction—and the occasional greater vigour of such germlings as compared with those formed by tetraspores, indicates a possible means of direct multiplication of the diploid



phase, perhaps supplemented by vegetative propagation. On the other hand, although Carter (1927) only met with diploid plants of *Padina pavonia* in British waters, he nevertheless found that reduction occurred during the formation of tetraspores. This shows that the foregoing explanation will not account for all the facts and suggests the presence of an undiscovered haploid phase which perhaps inhabits deeper water.

Among Sphacelariales pluri- and unilocular sporangia very commonly occur on distinct individuals, and this would appear to be the rule among the more specialized members. Reduction in the unilocular sporangium has been established for *Sphacelaria bipinnata* (Clint, 1927), *Halopteris scoparia* (Higgins, 1931a), and *H. filicina* (Mathias, 1935a), while the swarmers of the plurilocular sporangia have been shown to behave as gametes in *Cladostephus spongiosus* (Schreiber, 1931) and *Sphacelaria bipinnata* (Papenfuss, 1934). For the last, therefore, a definite isomorphic alternation is established, and there is considerable presumptive evidence of its occurrence also in other Sphacelariales. In certain species of *Sphacelaria* (Pringsheim, 1873, p. 170; Sauvageau, 1901, 1902), as well as in *Halopteris filicina* (Sauvageau, 1903, p. 419), plurilocular sporangia are found also on the diploid individuals, and in *S. bipinnata* (Clint, 1927, p. 17) their swarmers are diploid, fulfilling the same accessory role as in many Ectocarpales. This species would in fact appear to have a life-cycle very comparable to that of *Ectocarpus siliculosus*, and in this connexion it may be noted that Clint also records a fusion of the swarmers from the unilocular sporangia, although her figures are little convincing.

While an isomorphic life-cycle is probable among Tilopteridales, the reproductive processes are still so little known that a discussion of them in this article is not advisable.

#### HETEROMORPHIC ALTERNATION IN PHAEOPHYCEAE

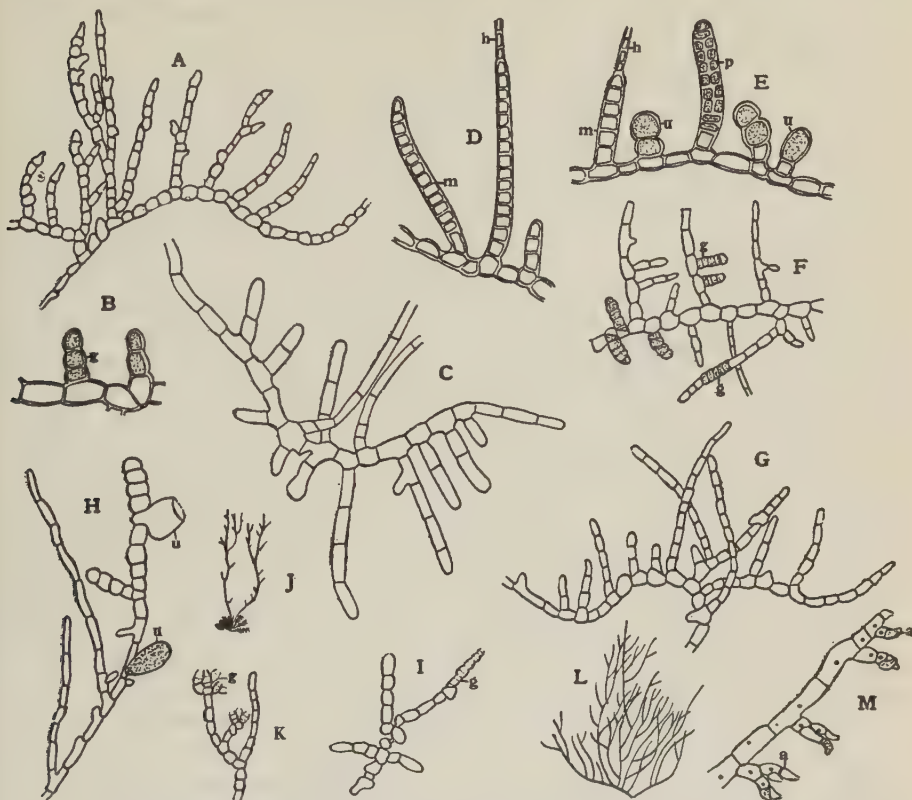
Heteromorphic alternation has now been demonstrated in six families of Ectocarpales, viz. among haplostichous forms in the Mesogloeaceae (*Castagnea virescens*, Parke, 1933; *Mesogloea vermiculata*, Parke, 1933; Kylin, 1933; *Sphaerotrichia divaricata*, Hygen, 1934<sup>1</sup>) and Spermatocnaceae (*Stilophora rhizodes*, Kylin, 1933; *Spermatocnus paradoxus*, Papenfuss, 1935a); among polystichous forms in the Punctariaceae (*Phloeospora brachiata*, Mathias, 1935), Asperococcaceae (*Asperococcus fistulosus*, Knight and Parke, 1931, p. 109<sup>2</sup>; *A. bullosus*, Knight, Blackler, and Parke, 1935), Encoeliaceae (*Soranthra ulvoidea*, Angst, 1926, 1927), and Dictyosiphonaceae (*Dictyosiphon foeniculaceus*, Sauvageau, 1917, 1929). The gametophyte is

<sup>1</sup> As *Nemacystus divaricatus*.

<sup>2</sup> The inclusion of *A. fistulosus* is based on the statements in the paper cited, although in a later one (Knight, Blackler, and Parke, 1935) reference is only made to the fusion of the swarmers from the unilocular sporangia. Kylin's data (1934, p. 13) indicate that there is a normal alternation.



always a minute filamentous plant, with the general characters of a small Ectocarpus or similar form (cf. Fig., c, j, k). The occurrence of reduction in the unilocular sporangium has been established in several of the species just listed, as well as in *Myriogloea Sciurus* (Parke, 1933, p. 30). There is an



A-C, *Spermatocnus paradoxus* Kütz.; A, plethysmothallus (from zoospore of plurilocular sporangium); B, C, gametophyte (from zoospore of unilocular sporangium), magnified more than twice as much as A. D-F, *Litosiphon pusillus* Harv.; D, germling from swarmer of plurilocular sporangium, with two young plants (*m*); E, ditto with a young plant (*m*), pluri- and unilocular sporangia; F, gametophyte (from swarmer of unilocular sporangium). G-I, *Protasperococcus myriotrichiiformis* Sauv.; G, plethysmothallus; H, ditto, threads with unilocular sporangia; I, gametophyte. J, K, *Stilophora adriatica* J. Ag., gametophyte; K, branch of same, with gametangia. L, M, *Carpomitra costata* (Stackh.) Batt.; L, prothallus; M, antheridial branch. *a*, antheridia; *g*, gametangia; *h*, hair; *p*, plurilocular and *u*, unilocular sporangia. (B, C, after Papenfuss; D-F after Kylin; the rest after Sauvageau.)

evident tendency among the more advanced Ectocarpaceae to eliminate to a large extent the accessory reproduction of the sporophyte by diploid swarmers which is frequent among the less specialized members. Plurilocular sporangia are infrequent on the diploid individuals of many of the species named above and in some of them are not yet recorded; even where they occur, plants with unilocular sporangia only are often reported.

Although the individual instances are not so clearly substantiated, presumptive evidence of the existence of heteromorphic alternation exists for a considerable number of other Ectocarpales.<sup>1</sup> In many of these the diploid plants bear both uni- and plurilocular sporangia, one or other type usually predominating at a certain stage of the life of the individual, although there is generally a longer or shorter period of overlap. Sometimes the two kinds of sporangia tend to occur on distinct plants, which in *Asperococcus*, for example, may show differences in size (Sauvageau, 1895; Kylin, 1907, p. 78; 1918, p. 15). When they occur on the same individual the plurilocular sporangia are generally formed before the unilocular ones, the most marked exception being afforded by *Leathesia difformis* (Sauvageau, 1925; Kylin, 1933, p. 64).

Small filamentous growths (Fig.) have been reared in cultures from the swarms of both uni- and plurilocular sporangia of many of these Ectocarpales. Such stages usually bear plurilocular sporangia (*g, p*), the swarms of which grow into similar ectocarpoid plantlets. There are commonly no noteworthy external differences between those produced from the swarms of the uni- and plurilocular sporangia of the macroscopic plant (cf. Fig., A and C, E and F), but it is highly probable that they contrast in chromosome-number. The stages reared from swarms of unilocular sporangia must be haploid; they are to be regarded as potential gametophytes and their plurilocular sporangia as gametangia. It is of importance in this connexion that, wherever the products of the swarms from the unilocular sporangia have been studied (Fig., B, F, I, K), the resulting prothalli bear *plurilocular sporangia only* (cf. Sauvageau, 1929, pp. 348, 353; Dammann, 1930, p. 11; Sauvageau, 1931, p. 145; Kylin, 1933, p. 32); moreover, there is no good evidence that they ever give rise directly to the adult thallus. The fact that the gametes, except in the species listed on p. 542, have shown no tendency for sexual fusion is no proof that such fusion does not occur in nature, since the conditions obtaining in laboratory cultures are only too likely to be abnormal. In this connexion attention may be drawn to the fact that Sauvageau (1929, p. 386) failed to obtain sexual fusion in *Asperococcus bullosus*, although this has since been observed (Knight, Blackler, and Parke, 1935): the same is true of *Mesogloea vermiculata* (cf. Kylin, 1933, p. 50, and Föyn, 1934, p. 7, with Parke, 1933, p. 36), *Castagnea virescens* (cf. Sauvageau, 1929, p. 284, and Kylin, 1933, p. 57, with Parke, 1933, p. 37), and *Spermatocchnus*

<sup>1</sup> viz. *Leathesia difformis* (Dammann, 1930, p. 11); *Castagnea Zosteræ* (? Sauvageau, 1927a, p. 411; Kylin, 1933, p. 63); *Chordaria flagelliformis* (Sauvageau, 1929, p. 269); *Liebmannia Leveillei* (? Sauvageau, 1929, p. 274); *Nemacystus ramulosus* (Sauvageau, 1931); *Stilophora adriatica* (Sauvageau, 1931, p. 145); *Myrionema strangulans* (? Kylin, 1934); *Desmotrichum undulatum* (? Kylin, 1933, p. 36); *Punctaria latifolia* (Sauvageau, 1929, p. 348; Ueda, 1930); *Myriotrichia repens* (Sauvageau, 1931); *Asperococcus echinatus* (? Sauvageau, 1928a); *Protasperococcus myriotrichiiformis* (Sauvageau, 1931, p. 81); *Litosiphon pusillus* (Sauvageau, 1929, p. 353; Kylin, 1933, p. 30); *Hapterophycus canaliculatus* (Hollenberg, 1941). Probably also in other Dictyosiphonaceae, apart from *Dictyosiphon foeniculaceus*.

*paradoxus* (cf. Sauvageau, 1931, p. 128, with Papenfuss, 1935). It is possible that extensive apogamous development of the gametes takes place in nature as it does in cultures, and that there is a succession of apogamous haploid prothalli, of which the ultimate ones produce gametes capable of sexual fusion and thus re-establish the diploid phase (Knight, 1931, p. 20); alternatively, the first-formed gametophyte may give rise to zygotes which develop a succession of similar diploid plethysmothalli. Perhaps these constitute some of the means of perpetuation during the unfavourable period of the year.

The filamentous growths, arising from the diploid zoospores of the plurilocular sporangia of the macroscopic plant, have never afforded any indication of sexual fusion on the part of the swarmers which they produce. Moreover, such stages (Fig., D, E) commonly develop the adult thallus (*m*) from their erect-growing branches and sometimes bear unilocular sporangia (Fig., E, H, U). The general features of these plethysmothalli and their status as arrested juvenile stages of the sporophyte have already been discussed (Fritsch, 1942, p. 406). The marked degree of similarity between them and the haploid gametophytic stages is only comprehensible on the assumption that the heteromorphic alternation of Ectocarpales is derived from an isomorphic one, the one generation (the sporophyte) having undergone elaboration of the erect system of the primary heterotrichous stage in diverse directions, whilst the other (the gametophyte) has remained at the level of the simple heterotrichous filament. The succession of plants formed from the diploid swarmers of the plurilocular sporangia of an *Ectocarpus siliculosus* or other unspecialized filamentous form are just as much plethysmothalli, but here, owing to the non-elaboration of the sporophyte, they are in general identical both with it and with the gametophyte in their morphological features.

Clearly marked heteromorphic alternation is met with in the Sporochneales (Sporochneus, Sauvageau, 1931, p. 123; Carpomitra, Sauvageau, 1926; Nereia, Sauvageau, 1927c), Desmarestiales, and Laminariales, in all of which the macroscopic plant bears unilocular sporangia only and the gametophyte exhibits oogamous reproduction by contrast to the isogamy prevalent among Ectocarpales. The structural features exhibited by the sporophytes in the first two orders represent particular variants on those met with in the haplostichous Ectocarpales, among which they were formerly classed (Oltmanns, 1922, p. 40); the Laminariales, on the other hand, must have a polystichous derivation. In view of the absence of a means of accessory reproduction of the sporophyte (apart from vegetative propagation), there is probably an obligate alternation between the two phases, as is clearly established for Desmarestiales (Schreiber, 1932) and Laminariales. As compared with the heteromorphic Ectocarpales there is thus a simplification and stabilization of the life-cycle.

Although in the three orders under consideration the sporophyte shows no traces of heterotrichy, the gametophytes do not differ in any fundamental respect in vegetative characters from a simple Ectocarpus (cf. Fig., L).



In view of this and of the obvious parallel in vegetative construction of the sporophyte of Desmarestiales and Sporochnales with that found among the more advanced Ectocarpales, there is every reason to believe that the loss of the prostrate system in the early development of the sporophyte is secondary and that, as in the heteromorphic Ectocarpales, the two generations have diverged from isomorphic types.

Such an hypothesis is more difficult to apply to the Laminariales, in view of the usual high degree of specialization attained by the sporophyte in this order. The early stages of development of Chorda, however, show many points of contact with those of the more specialized polystichous Ectocarpales, with which Chorda was indeed classed prior to the discovery of its gametophytes. Even in the more specialized Laminariales there is a definite, though brief, filamentous stage in the early development of the zygote which nearly always undergoes a series of transverse divisions before the commencement of septation in other planes. In cultures of *Laminaria saccharina* Pascher (1918) has, moreover, recorded a precocious development of zoospores in embryos in which surface-development was just beginning, as well as in some which were still in the 2-8-celled filamentous stage. These observations suggest that, under certain conditions, production of zoospores might take place without the formation of an elaborate sporophyte, and they lend support to the view of an ultimate isomorphic origin, even for the sharply distinct generations of Laminariales. The strongest argument in favour of this view is, however, the undoubted isomorphic derivation of elaborate types with heteromorphic alternation among Ectocarpales. The Laminariales may be regarded as exhibiting to an extreme the elaboration of the sporophyte so patent in the latter order, although the filamentous heterotrichous habit has been retained by the gametophyte.

#### MODIFIED TYPES OF LIFE-CYCLE AMONG ECTOCARPALES

Some reference has already been made to various ways in which the life-cycle may be curtailed among Ectocarpales (p. 540), but these are deviations in species which under other circumstances exhibit a full phase-alternation. There are, however, several Ectocarpales in which, so far as present evidence goes, such an alternation appears to be lacking. Thus, in *Striaria attenuata* (Kylin, 1934, p. 15) and *Dictyosiphon Chordaria* (Föyn, 1934, p. 7), in which the plants produce unilocular sporangia only, the filamentous growths, arising from their swarmers in cultures, after some time develop an adult thallus from the branches of the erect system. In the latter species these growths may also bear unilocular sporangia, from whose swarmers four successive generations of such filamentous stages have been obtained.

These two species are usually regarded as diploid forms in which there is no reduction in the unilocular sporangium (Kylin, 1934, p. 17; Knight, Blackler and Parke, 1935, p. 87) and, on the available evidence, this would



appear to be the only valid interpretation, although a cytological investigation is clearly indicated. They would exemplify an abbreviation of the life-cycle analogous to that found in certain Florideae (*Lomentaria rosea*, p. 554) and possibly also occurring in a somewhat different way in Dictyotales (p. 541). It should be noted that some of the allies of *Striaria attenuata* and *Dictyosiphon Chordaria* show a normal alternation. Some light is shed on the possible mode of origin of this condition among Ectocarpales by Hollenberg's (1941, p. 677) observations on Hapterophycus, according to which certain of the unilocular sporangia probably show a non-occurrence of meiosis. The swarmers produce two kinds of germings, of which the larger bear no reproductive organs and appear to develop direct into the macroscopic plant. Other instances of a condition, similar to that found in the two species above discussed, are probably afforded by *Pylaiella rupicola* (Kylin, 1937, p. 6; Levring, 1940, p. 33) and *Asperococcus compressus* (Reinke, 1878a; Sauvageau, 1929, p. 386) where, however, plurilocular sporangia have also been recorded.

It has long been known that the thalli of *Scytosiphon*, *Petalonia* (Phyllitis), and *Colpomenia* bear plurilocular sporangia only (Sauvageau, 1929, p. 402). Fusion of the swarmers has repeatedly been reported in *Scytosiphon Lomentaria* (Berthold, 1881, p. 407; Kuckuck, 1898; 1912a, p. 160; Frye, 1930; Abe, 1935, p. 333) and once in *Petalonia zosterifolia* (Kuckuck, 1912a, p. 162) and *Colpomenia* (Kunieda and Suto, 1938). Others (Sauvageau, 1929, p. 332; Dammann, 1930, p. 12; Kylin, 1933, p. 47) have found that the swarmers of *Scytosiphon* give rise, without fusion, to a system of creeping filaments which may perhaps reproduce by plurilocular sporangia, but more usually unite to form a disc and regenerate a new plant (cf. Fig. 2, B in Fritsch, 1942). For *Petalonia debilis* (Sauvageau, 1929, p. 323) and *P. Fascia* (Kylin, 1933, p. 44) it has likewise been shown that the swarmers can give rise to filamentous stages producing new plants directly, and according to Sauvageau (1927b, p. 350) this is also true of *Colpomenia*.

It is difficult to reconcile the data on direct development of a new thallus from the growths formed by the swarmers of the plurilocular sporangia with the records of their sexual fusion. It suggests that there may be several types of plurilocular sporangia in these genera, and this finds some support in the recent work on *Colpomenia* (Kunieda and Suto, 1938). Kylin (1933, p. 46) is of the opinion that the three genera under discussion possess diploid thalli which no longer produce unilocular sporangia and that the recorded fusions are due to misinterpretation. The possibility, however, that reduction might take place during the development of certain of the plurilocular sporangia cannot altogether be dismissed (cf. also Kniep, 1928, p. 256). Similar conditions occur in *Giraudya* (Sauvageau, 1927, p. 46) and among the species of *Stictyosiphon* (Kuckuck, 1912a, p. 164; Sauvageau, 1929, p. 306). It is noteworthy that the majority of the genera considered in this section belong to the polystichous Ectocarpales.

## THE LIFE-CYCLE IN THE RHODOPHYCEAE

(a) *Bangiales*

The Bangiales, like the Nemalionales, are probably haploid forms in which reduction takes place during the first two divisions in the zygote; this has been rendered probable for *Porphyra* by Dangeard (1927, p. 223). Sexual plants appear to occur mainly during the cold season, whilst at other times of the year the thalli, if present at all, often bear monosporangia only. There can be no doubt that the latter constitute mere accessory organs of reproduction, since the monospores of the Bangieae (*Bangia*, *Porphyra*) develop direct into a new plant (Kylin, 1922, p. 5; Dangeard, 1927, pp. 205, 225; 1931; Chemin, 1937, p. 228). The fate of the carpospores is not altogether clear. According to Kunieda (1939, p. 386) those of *Porphyra tenera* remain dormant throughout the warm season and produce new plants in the late autumn. Earlier investigators,<sup>1</sup> following the development of the carpospores of *Bangia* and *Porphyra* in cultures, concluded that they gave rise to branched filamentous stages, from which supposed monospores were liberated; Kunieda (p. 391) regards such stages as pathological. Rees (1940; 1940a, p. 429), who studied the life-cycle of *P. umbilicalis* in nature, however, arrived at much the same result. He found that the carpospores grew into short, little-branched filaments, lasting only for a few weeks and multiplying by means of monospores; ultimately these stages developed into the *Porphyra*-thallus (cf. also Ueda, 1929). There is thus considerable evidence that, in the northern hemisphere at least, the Bangieae persist during the unfavourable season by means of minute filamentous stages, analogous to the plethysmothalli of many Ectocarpales, though probably haploid.

(b) *Nemalionales*

Whilst in the Bangiales the entire zygote subdivides to form carposporangia, the Nemalionales produce the latter on branched filamentous outgrowths (gonimoblasts) arising from the fertilized carpogonium. The contrast has perhaps not been sufficiently emphasized. The life-cycle of Bangiales resembles that of the haploid Chlorophyceae and, where the number of carpospores produced from the zygote exceeds four, there is particular resemblance to that of Coleochaete. The condition prevalent in Nemalionales engenders the development of a distinct morphological phase, an elaboration of the zygote, and thus affords opportunity, if the place of meiosis be shifted, for the origin of a dependent sporophyte of a type contrasting markedly with the ordinary gametophyte. It is perhaps open to question whether the structures customarily so called are correctly designated carpospores among

<sup>1</sup> Janczewski, 1873, pp. 244, 249; Thuret and Bornet, 1878, p. 61; Reinke, 1878b, p. 278; Berthold, 1882, p. 19; Yendo, 1919, p. 81; Okamura, Onda, and Higashi, 1920; Kylin, 1922, p. 6; Grubb, 1924, p. 229; Dangeard, 1931.

Bangiales. They would appear to be homologous with the primary products of segmentation of the zygote of a *Batrachospermum* or *Nemalion*, which in *Nemalionales* are not liberated but develop *in situ* into the filamentous gonimoblasts; on such an interpretation the latter would be homologous with the filamentous stages formed from the 'carpospores' of Bangiales and the carpospores of *Nemalionales* would be homologous with the monospores borne upon the former.

Since the time when Svedelius (1915, p. 32) demonstrated that in *Scinaia* reduction occurs during the first two divisions of the zygote-nucleus, this has been proved also for diverse other *Nemalionales* (Kylin, 1916a, p. 267; 1917, p. 161; Cleland, 1919, p. 338; Kylin, 1928, p. 9; Svedelius, 1933, pp. 27, 44), and it cannot be doubted that this is the normal condition throughout the order. There are thus two contrasting haploid phases and the life-cycle is haplobiontic; the zygote, as in haploid forms, represents the only diploid stage.

Increasing specialization is evident, both in the uni- and multi-axial *Nemalionales*. In each series the less specialized members lack definite nutritive cells in the carpogonial branch, although these are differentiated in the uniaxial *Naccariaceae* (Zerlang, 1889, pp. 394, 401; Kylin, 1928, p. 13) and *Bonnemaisoniaceae* (Kylin, 1916; 1928, p. 24; Svedelius, 1933) and in the multi-axial *Chaetangiaceae* (Svedelius, 1915; Martin, 1939, p. 119); it is only in the last two families, moreover, that a sharply circumscribed envelope is formed around the gonimoblast leading to the formation of a true cystocarp. In *Acrochaetium*, *Batrachospermum*, and *Lemanea* there is no fusion of the fertilized carpogonium with other cells, such as obtains in the less specialized multi-axial forms (*Nemalion* and other *Helminthocladia-ceae*), where cells of the carpogonial branch thus provide nutriment for the developing gonimoblast. In *Naccariaceae* and *Bonnemaisoniaceae*, however, the nutritive cells occur as outgrowths from the hypogynous cell of the carpogonial branch, and this is also essentially so in *Scinaia* (*Chaetangiaceae*); in most of these there are complex fusions after fertilization.

The gonimoblasts usually originate directly from the carpogonium, but in *Scinaia*, *Chaetangium*, and *Asparagopsis* the diploid nucleus passes into a hypogynous cell, and it is from this that the gonimoblast arises. The post-fertilization behaviour of the diploid nucleus in these genera is analogous to that shown in many diplobiontic *Florideae*, and there seems no valid reason why the hypogynous cell into which it passes should not be termed an auxiliary cell (Svedelius, 1933, p. 49; Martin, 1939, p. 139; cf., however, Kylin, 1930, p. 92; 1935, p. 140). It would seem that the more advanced haplobiontic *Florideae*, despite the fundamental difference in their life-cycle, show many of the features that characterize the diplobiontic type, and it is of importance in this connexion that the vegetative structure of the genera involved also affords evidence of considerable specialization.

Accessory reproduction of the gametophyte by means of monospores takes



place in several of the less specialized Nemalionales, although unknown in Lemanea, Nemalion, Naccariaceae, and Bonnemaisoniaceae. Such monosporangia are particularly frequent in *Acrochaetium* (Chantrynsia), some individuals often bearing them alone, although they commonly occur also on plants with sex organs and carposporangia. Several species of this genus (*A. Daviesii*, *A. Thureti*, *A. virgatulum*) bear tetrasporangia as well as monosporangia, both usually occurring on the sexual plants (Boergesen, 1903, p. 351; Kylin, 1907, p. 118; Rosenvinge, 1909-31, p. 85). On the other hand, in *A. violaceum* (Drew, 1935) and probably in *A. efflorescens* (Lehman, 1902; Kylin, 1906; Rosenvinge, 1909-31, p. 85), there are distinct tetrasporic and sexual individuals which may also bear monosporangia, although in *A. efflorescens* these often occur on distinct plants. The tetrasporic and sexual plants exhibit a certain seasonal alternation, since the former occur mainly during the colder and the latter during the warmer months (Rosenvinge, 1909-31, p. 137; Drew, 1935, p. 448). The tetrasporangia are always cruciately divided and such sporangia are the only reproductive organs at present certainly established for *Rhodochorton*.

The question whether *A. violaceum* and *A. efflorescens* might be diplobiontic can only be settled by cytological investigation, but in face of the known facts it seems improbable. Although no data as to the place of reduction are available for *Acrochaetium*, it is generally assumed that it occurs, as in other Nemalionales, during the first two divisions of the zygote nucleus. In those species which bear tetra- and monosporangia on plants with functional sex organs reduction in the tetrasporangium as a normal phenomenon is inconceivable. The occasional association of monosporangia with tetrasporangia on the non-sexual plants of *A. efflorescens* and *A. violaceum* renders it equally improbable that the tetrasporangia are the seat of a reduction division. With our present knowledge it seems most plausible to regard these tetrasporangia merely as divided monosporangia (cf. also Kniep, 1928, p. 219), and in this connexion attention may be drawn to the presence in certain species of bispores (Boergesen, 1909, p. 179; 1915, p. 43; 1927, p. 20) and polyspores (Howe, 1914, p. 88; Boergesen, 1937, p. 38).

Cruciate tetrasporangia are also found in many species of *Galaxaura*, either on the sexual plants or more usually on distinct individuals which often differ markedly from the sexual ones (Howe, 1917; Weber van Bosse, 1921, p. 209; Boergesen, 1927, p. 65). The possibility that this advanced member of Nemalionales might be diplobiontic is perhaps greater, but can only be settled by a cytological investigation.

Whether any of the Nemalionales have progressed to the diplobiontic condition or not, certain members of this order provide evidence of an advance in another direction. In *Liagora tetrasporifera* (Boergesen, 1927, p. 39; Kylin, 1930, p. 9) and *Helminthocladia Hudsoni* (Feldmann, 1939a, p. 94) the terminal cells of the gonimoblasts form cruciate tetraspores instead of the carpospores produced in other species of these genera. It has been con-



cluded that, in the species named, reduction takes place during the formation of the tetraspores, although this need not necessarily be so. If, however, cytological study prove this assumption to be correct, postponement of meiosis within the domain of a single genus would have been demonstrated. Moreover, the condition above referred to, viz. the development of a sporophytic phase parasitic on the gametophyte, would have been realized, and we should have a true antithetic alternation in the English sense.

In members of *Bonnemaisoniaceae* Feldmann (1939*b*) has recently reported the production from the carpospores of an independent generation bearing tetrasporangia with tetrahedral spores. That of *Bonnemaisonia asparagoides* (Feldmann and Mazoyer, 1937) is stated to be identical with Batters' *Hymenoclonium serpens* (1895, p. 318), while that of *Asparagopsis armata* (Feldmann, 1939) is represented by the alga *Falkenbergia*. Should these observations be corroborated, they would illustrate another variant in the life-cycle, although in view of the data furnished by Svedelius (1933) there can be no doubt that both species are haplobiontic. They would, however, provide added evidence for the view that the occurrence of tetrasporangia in *Nemalionales* is not necessarily linked with a reduction division.

### (c) *The diplobiontic Florideae*

Cytological corroboration of the existence of distinct diploid asexual and haploid sexual phases has now been obtained in a considerable number of diplobiontic *Florideae*. In *Cryptonemiales*, *Gigartinales*, and *Rhodymeniales* there is usually, despite occasional exceptions (cf. Kniep, 1928, p. 223), a sharp segregation of sporangia and sex organs on separate individuals, although among *Ceramiales* departures from this rule are relatively frequent. A regular alternation of tetrasporic and sexual individuals has commonly been recorded in nature and has been established experimentally by Lewis (1912) for a number of *Florideae*. These facts imply the widespread occurrence of an isomorphic alternation of haploid and diploid phases.

Among *Ceramiales* procarys or more rarely antheridia commonly occur side by side with sporangia on the same individual. The first instance of this kind to be fully studied was that of *Nitophyllum punctatum* (Svedelius, 1914), in which plants bearing mature cystocarps often possess sporangia as well. The latter are grouped around rudimentary procarys which suggests a possible correlation between sporangium-formation and arrest of procarys. As in other species of *Nitophyllum*, the sporangial initial is multinucleate, although ultimately only one nucleus survives. On sexual plants this nucleus fails to divide and the entire contents of the sporangium are liberated as a single monospore possessing the haploid number of chromosomes, although the carpospores of such plants are as usual diploid. The nuclei of sporangia, occurring on male plants of *Polysiphonia violacea* (Yamanouchi, 1906, p. 425) and *Griffithsia globifera* (Lewis, 1909, p. 672), likewise usually remain undivided, although cleavage furrows may appear within the protoplast without

reaching the centre; the occasional nuclear divisions afford no evidence of meiosis. In *Polysiphonia urceolata* Rosenvinge (1909-31, p. 410) also records small undivided sporangia on plants with mature cystocarps. It is thus probable that the sporangia found on sexual plants produce haploid monospores which may play a role in its accessory reproduction (cf. also Lewis, 1909, pp. 671, 682; Svedelius, 1937, p. 6).

Asexual plants bearing normal tetrasporangia side by side with arrested procarps and antheridia have been repeatedly recorded in *Spermothamnion* (Lewis, 1909, p. 683; Kylin, 1916b, p. 86; Schussnig and Odle, 1927, pp. 224, 250). In certain forms of this genus, on the other hand, typical tetrasporangia occur on plants with apparently functional sex organs. Schussnig and Odle's data (1927, p. 226) in support of the non-occurrence of reduction in such sporangia are scarcely convincing and are, moreover, at variance with the conclusions reached by Drew (1934) in *S. Turneri*. She provides clear evidence of the occurrence of meiosis in the sporangia of plants bearing developing gonimoblasts and mature fruits, as well as rare antheridia. Such individuals are therefore diploid ( $2n = 60$ ) and their tetraspores haploid, although so far no haploid plants of this species have been found in nature. Drew also provides data (1934, p. 559) to show that the procarps of the plants in question are diploid and their gonimoblasts tetraploid, although the occurrence of fertilization is not clearly established and a large number of the procarps fail to develop further. There is likewise some evidence of the occurrence of triploid carpospores, such as might result from the fertilization of a diploid carpogonium by a haploid spermatium. So far it has not been possible to secure germination of either the tetraploid or the triploid carpospores. Mathias (1928, p. 20) found tetrasporangia exhibiting reduction, together with fruits, on the same individual of *Callithamnion brachiatum*, although here the carpospores are stated to be diploid (Mathias, 1932), and the possibility of an apogamous development of the female organ on such plants must not be overlooked.

The haplo- and diplobiontic Florideae are doubtless related, and the view has been frequently expressed that the latter originated from forms with a haplobiontic life-cycle (cf. Yamanouchi, 1906, p. 435; Lewis, 1909, p. 682; Svedelius, 1915, p. 43; Kniep, 1928, p. 217; Svedelius, 1931, p. 41), probably as a result of a sudden mutation (Svedelius, 1927, p. 369). The state of affairs would be similar to that postulated among Chlorophyceae (p. 536). The majority of the haplobiontic forms exhibit a simpler vegetative structure than the diplobiontic ones and moreover afford marked evidence of heterotrichy (Fritsch, 1942, p. 400), and there is little to justify the hypothesis of a possible reduction from diplobiontic types (p. 557). The occasional occurrence of tetrasporangia on the sexual plants of *Acrochaetium* (p. 550) warrants the supposition that they originated among the ancestors of the diplobiontic Florideae by division of the contents of monosporangia prior to the inception of a diplobiontic life-cycle. It may be conjectured that, with the

first establishment of isomorphic alternation by the postponement of the reduction division from the zygote to the tetrasporangium, each of the two phases still bore tetrasporangia, although reduction will have been associated with the sporangia of the one phase only; it is also possible that this phase for a time still possessed functional or functionless sex organs. The persistence of abortive sex organs on the diploid and of monospore-producing sporangia on the haploid phase in certain Ceramiales would on this view be tokens of an ancestral condition and would mark a more primitive state than the sharp individualization of the two phases that characterizes many diplobiontic Florideae. It is noteworthy in this connexion that the simultaneous presence of sex organs and sporangia is more frequent in the relatively primitive Ceramiaceae and in Polysiphonia than in the more specialized members of Ceramiales.

In the light of the hypothesis just stated *Spermothamnion* may perhaps be regarded as a genus in which sex organs have persisted on the diploid stage and in certain, probably rare, instances remained functional (cf. also Drew, 1934, p. 567), although they may also have secondarily reacquired their former function. However that may be, the possibility for the origin of tetraploid and triploid races afforded by such a state of affairs is of considerable importance, and the further investigation of these matters will be awaited with interest.

It may be noted that the primitive diplobiontic type postulated above, if sexual fusion occurred, would provide opportunity for the origin of tetraploid forms, and this perhaps explains the fact that the majority of the investigated diplobiontic Florideae have twice as many chromosomes as the haplobiontic ones (Knip, 1928, p. 219). Further increases in chromosome-number were probably prevented by resulting sterility of the sexual cells so that the sex organs on the diploid (tetraploid) individuals became functionless and gradually aborted. The condition seen in *Liagora tetrasporifera* and *Helminthocladia Hudsoni* is not necessarily intermediate between the haplo- and diplobiontic types, since, when immediate meiosis failed to occur in the zygote, reduction might ensue at any place in the resulting diploid phase. The two species mentioned would illustrate one solution, the diplobiontic Florideae another.

The Ceramiaceae are remarkable for the frequent occurrence on the asexual, but never on the sexual, individuals of special types of reproductive organs. It is beyond the purpose of this paper to consider these in detail, but the rather widely distributed polypores (see Westbrook, 1930, p. 360) merit some attention. They are also recorded in *Gonimophyllum* (Setchell, 1923, p. 394) and in *Chylocladia* (Miranda, 1931). In the former, as well as in *Pleonosporium* and in *Spermothamnion Snyderae* among Ceramiaceae, they replace the tetrasporangia. In the two last, in which the polypores occur in multiples of 4 (Miranda, 1931, p. 194; Drew, 1937), there is evidence of the occurrence of meiosis during the first nuclear divisions in the sporangium so that the polypores represent the haploid reproductive cells of the asexual



phase. In most Ceramiaceae, however, the polysporangia are accompanied by tetrasporangia, although the former tend to predominate in certain (colder ?) habitats. In the absence of a cytological investigation it is uncertain whether they represent tetrasporangia in which division has progressed beyond the usual 4-celled stage or whether they are accessory reproductive organs producing diploid spores. In *Plumaria elegans* they are usually found on distinct plants, and particular interest attaches to the recent demonstration by Drew (1939) that these individuals are triploid and that no reduction occurs in the formation of the polyspores. This species possesses haploid sexual and diploid asexual phases, the latter with tetrasporangia, so that there would appear to be a normal diplobiontic cycle. The mode of origin of the triploid plants is unknown, although the probable occurrence of triploid carpospores in *Spermothamnion Turneri* indicates one way in which this condition could arise.

More pronounced deviations from the diplobiontic life-cycle than those above discussed are known for certain Florideae. Thus, in *Trailiella intricata*, *Antithamnionella sarnensis*, *Dasya ocellata*, and *Lomentaria rosea* sexual individuals have so far not been found. In the last, a northern species always inhabiting water of considerable depth, it has been shown (Svedelius, 1937) that no reduction occurs during tetraspore-formation. Comparison with other Lomentarias having a normal life-cycle indicates that the plants of *L. rosea* are diploid so that in this species the sexual phase appears to have been suppressed. Segawa (1936, p. 185), it is true, has described cystocarps, but it seems probable that his plants belong to a different species. The modification of the life-cycle shown by *L. rosea* is analogous to that above noticed in certain Ectocarpales (p. 546). It is noteworthy that in *Dasya ocellata* also Westbrook (1935, p. 571) failed to obtain good evidence of the occurrence of reduction in the tetrasporangium.

In *Agardhiella* the entire contents of the sporangium commonly grow out as a single structure (Osterhout, 1896, p. 419); for a time the products of division of the individual spores remain distinguishable, but by degrees the limits become obscured. The base of the single proliferation that thus arises is embedded in the parent thallus and the lower cells give rise to penetrating rhizoids. These growths bear male or female sex organs or more rarely tetrasporangia; the presence of the last may imply absence of reduction. Other similar instances are reported in *Cystoclonium* (cf. also Rosenvinge, 1909-31, p. 593), *Gracilaria*, *Champia parvula* (Nott, 1896, p. 164), and *Lomentaria rosea* (Svedelius, 1937, p. 19); in the last the proliferation results from the diploid tetraspores. Such phenomena merit investigation in detail. They are of interest since, when the growths under discussion bear sex organs, the sexual plant is parasitic on the asexual one, a condition which is the converse of that found in the Gigartinales about to be discussed.

It is in certain members of this order that the most fundamental changes in the life-cycle have been demonstrated. Although diverse species of



Phyllophora follow a normal diplobiontic life-cycle (cf. Kylin, 1928, p. 54), others show striking abbreviation. In *P. Brodiaei* (Rosenvinge, 1929, p. 13; 1909-31, p. 525) spermatia have not been observed on the trichogynes and the procarys are sometimes degenerate, but Kylin (1930, p. 28) records fusion of the carpogonium with the adjacent auxiliary cell and regards this as evidence of fertilization. Many of the branched gonimoblast-threads, arising from the auxiliary cell, grow towards the exterior and, piercing the surface, initiate a slowly maturing nemathecium which produces numerous rows of sporangia, forming cruciate tetraspores. These nemathecia were long thought to belong to an independent parasite (*Actinococcus subcutaneus*), but it now seems beyond doubt that they represent the asexual phase of *Phyllophora Brodiaei*, the carposporangial stage being suppressed and the gonimoblasts giving rise directly to the nemathecial sporophyte. Claussen (1929) produces rather inconclusive evidence that the nuclei of the latter are diploid, and the cytological aspects of the life-cycle require further study. The tetraspores, however, develop into young plants (Rosenvinge, 1909-31, p. 530) which are sufficiently like the mature gametophyte to complete the picture of the life-cycle.

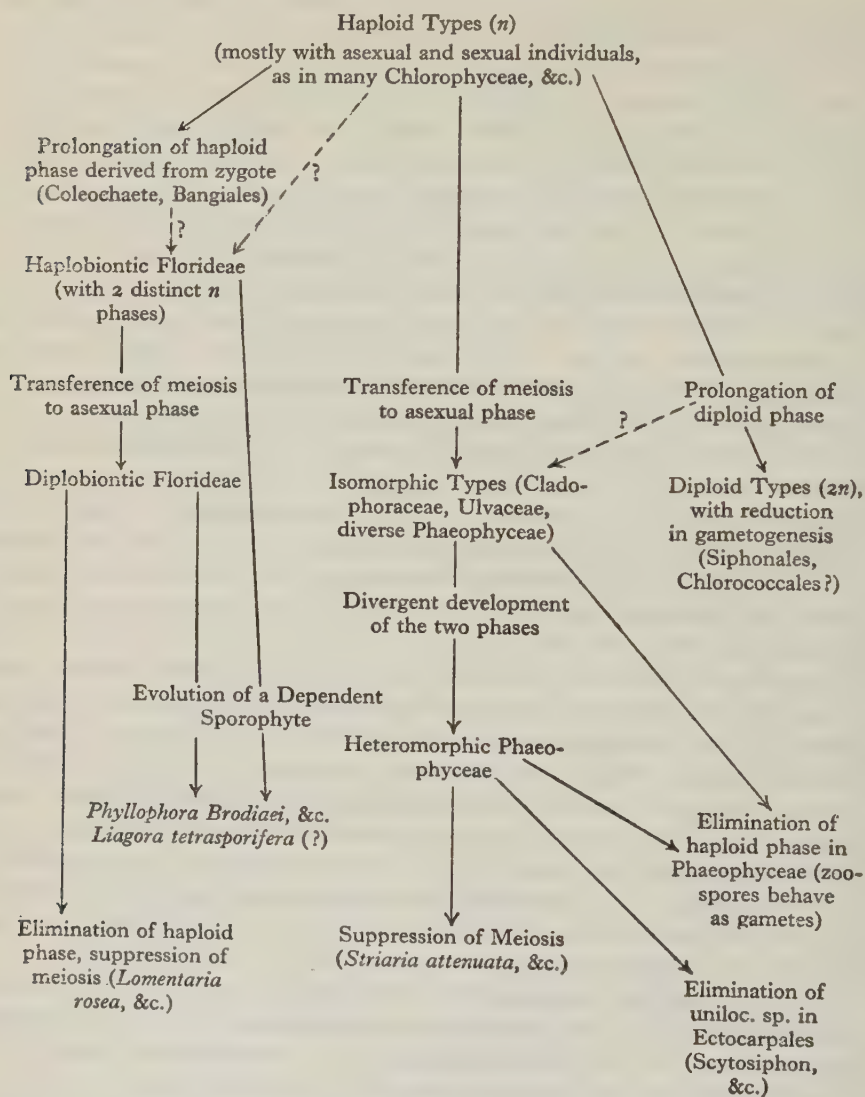
Similar 'parasites' are recorded in several other species of *Phyllophora* (Phillips, 1925, p. 251), in all of which no asexual individuals are known, while the 'parasite' produces only tetraspores. There is thus considerable presumptive evidence that these species possess an abbreviated life-cycle like that of *P. Brodiaei*.

An analogous condition is known among the species of *Gymnogongrus*. *G. norvegicus* is probably a normal diplobiont, since it possesses separate cystocarpic and tetrasporic individuals, but in many species no cystocarps have been found (Phillips, 1925, p. 250). In two of these (*G. Griffithsia*, *G. platyphylla*) the 'parasites' (*Colacolepis aggregatus*, *Actinococcus chiton*) have been shown to represent the sporophytic phase (Gregory, 1934, p. 532; Doubt, 1935) which originates from threads growing out from the auxiliary cell of the procary; it produces either tetraspores or monospores (Chemin, 1933). These two species are possibly haploid throughout their life, since antheridia have not been found.

The most complete condensation of the life-cycle is met with in *Ahnfeltia plicata*, in which the only reproductive structures that have been recorded are the monospore-producing nemathecia, until recently usually regarded as belonging to a parasite (*Sterrocolax decipiens*). It is now, however, clear that these nemathecia result from a mere proliferation of the cortex of the *Ahnfeltia* thallus (Rosenvinge, 1931; 1909-31, p. 560; Gregory, 1934, p. 534). The monospores produced by them grow into young *Ahnfeltia* plants (cf. also Chemin, 1930). No recognizable procarys have been found.

*Ahnfeltia* thus provides a further stage in the reduction-series exemplified by *Phyllophora Brodiaei* and *Gymnogongrus*. The condition found in the former shows a marked degree of correspondence with that above described

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 for *Liagora tetrasporifera* and *Helminthocladia Hudsoni* (p. 550) and affords  
 a striking example of convergent development. In either instance, although



*Scheme of the principal types of life-cycle among the Algae*

from an altogether different starting-point, a probable diploid phase parasitic upon the haploid one is attained. The relation of the one to the other is comparable with that which obtains in Bryophyta.

Such a form as *Gymnogongrus Griffithsiae*, which is possibly haploid throughout its life-cycle, is relevant in a consideration of the relative status

of the haplobiontic and diplobiontic Florideae. The former have sometimes been held to be derived from diplobiontic forms (Church, 1919), but if this were so a general simplification must be assumed to have taken place concurrently, since in almost every respect most haplobiontic Florideae are less specialized than the diplobiontic forms (cf. also Svedelius, 1931, p. 45). There is, moreover, presumptive evidence of a postponement of the reduction division in species of *Liagora* and *Helminthocladia* (p. 551), whereas here are no facts supporting a transference of the seat of reduction from the tetrasporangium to the zygote among Gigartinales. The Phaeophyceae illustrate how tenaciously the place of the reduction division may be confined to a definite type of reproductive organ (the unilocular sporangium) when once established. Moreover, among Chlorophyceae the data support an analogous sequence from haploid to isomorphic types.

#### GENERAL CONCLUSION AND SUMMARY

Ignoring for the time being the many deviations that occur in a limited number of forms, the majority of the Algae that possess sexual reproduction exhibit one of three types of life-cycle—haploid, isomorphic, heteromorphic. For the present purpose the haplobiontic Florideae can be grouped with the haploid, the diplobiontic Florideae with the isomorphic forms. In the foregoing pages support has been given for the view that the haploid life-cycle represents the primary condition and that the isomorphic type found in diverse Chlorophyceae, many Phaeophyceae, and the diplobiontic Florideae originated from it. How this fundamental change, involving transference of meiosis from the zygote to the time of spore-production in the asexual phase, was brought about is unknown, but it is generally believed to have taken place in one step. It should be realized that the majority of haploid types found at the present day reproduce often for long periods by purely asexual means; in other words, they possess both asexual and sexual individuals, although with the same chromosome-content, so that the forward step resulting in an isomorphic phase-alternation consisted mainly in the shifting of the place of reduction. On the other hand, especially among Siphonales, the primary step in the evolution of isomorphic from haploid types may have been the production of a diplont (p. 537), and such a derivation is also possible for other isomorphic forms, although improbable among Florideae.

The heteromorphic alternation met with in many Ectocarpales is clearly derived from an isomorphic one by elaboration of the sporophyte, while the gametophyte remains a simple heterotrichous filament. The early stages of development of the sporophyte are in all essential respects morphologically identical with the gametophytes (Fig. D-F). In Sporochneales, Desmarestiales, and Laminariales this fundamental similarity is obscured, since, as in most advanced heterotrichous types, the prostrate system of the sporophyte has disappeared. There is, however, every reason to believe that these Phaeophyceae



also are descended from forms with an isomorphic alternation, since in vegetative features the heterotrichous gametophytes essentially resemble those of the Ectocarpales. The conclusion is therefore reached that, wherever heteromorphic alternation occurs among the Algae, it is derived from the isomorphic type (cf. also Cutleria, Fritsch, 1942, p. 409). The sequence of life-cycle—haploid-diploid (?)—isomorphic-heteromorphic—is that which is fundamental for the Algae as a whole.

The principal deviations from these basic types are indicated in the scheme on p. 556. The most significant is that leading to the haplo- and diplobiontic life-cycles of Florideae in which the zygote gives rise to a dependent generation, haploid or diploid as the case may be, which contrasts sharply in character with the normal gametophyte or sporophyte. These are the only instances among Algae in which a truly antithetic generation is produced. Other modifications are expressions of the general plasticity that is evident especially among the less specialized Phaeophyceae. The short-circuiting of the life-cycle by the adoption of sexuality on the part of the swimmers of the unilocular sporangium is particularly noteworthy.

It is remarkable that, despite the many modifications, the place of the reduction division in iso- and heteromorphic forms is rigidly maintained. The accessory reproduction characteristic of the less specialized haploid types, and found also among the iso- and heteromorphic Phaeophyceae, is lacking in most of the more advanced members of all classes with a definite phase-alternation.

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<sup>1</sup> This list is not intended to be an exhaustive bibliography of publications dealing with the life-cycle of the Algae, but includes only those to which reference is made in the text.



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# Chrooderma, a new Genus of Subaerial Algae

BY

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With three Figures in the Text

THE alga about to be described was sent to me in 1932 by the Rev. P. G. M. Rhodes. It had been discovered on dead stems of '*Rubus fruticosus*' in the Bonython Plantations, Bochym, West Cornwall, in April of that year, while searching for Pyrenomycetes. Unfortunately only dried material was received and, although this sufficed to bring out many of the structural features, it was inadequate for the study of the details of cell-structure and of the processes of reproduction. I have so far been unsuccessful in finding the alga elsewhere. It is possibly confined to the damper areas along the west coast which I have not visited in recent years.

The collector concluded that the alga was *Phycopeltis epiphyton* Millardet (1870), with which indeed certain stages exhibit some resemblance, but a detailed study of the material has shown very significant differences from *Phycopeltis* which render a reference to that genus impossible. Although no doubt a member of the Trentepohliaceae, the alga is distinguished by having both epiphytic and endophytic systems and more particularly by the fact that the former becomes several-layered in the older parts. Although various features require further elucidation, there can be no doubt that the alga affords a new example of thallus-construction among subaerial Algae.

It forms small, pale yellow-brown crusts which occupy a large part of the surface of the bramble shoots (Fig. 1, A). When isolated the growths are often approximately circular (Fig. 1, B) and 0.5–2 mm. in diameter, but in places they are confluent into larger irregular expanses and on parts of some of the shoots there is an almost continuous covering formed by the alga. All except the very smallest growths usually consist of several layers of cells over most of their extent, although a larger or smaller part of the margin is generally one-layered (Fig. 1, B). I was for some time in doubt whether the several-layered character might not be due to successive one-layered plants overgrowing one another. It is possible that such overgrowth occurs, although on the whole contiguous plants do not overlap. There can, however, be no question that the several-layered condition of the majority of the crusts is due to quite different causes. Many of them, which are quite widely separated from adjacent plants, show clearly a one-layered edge and

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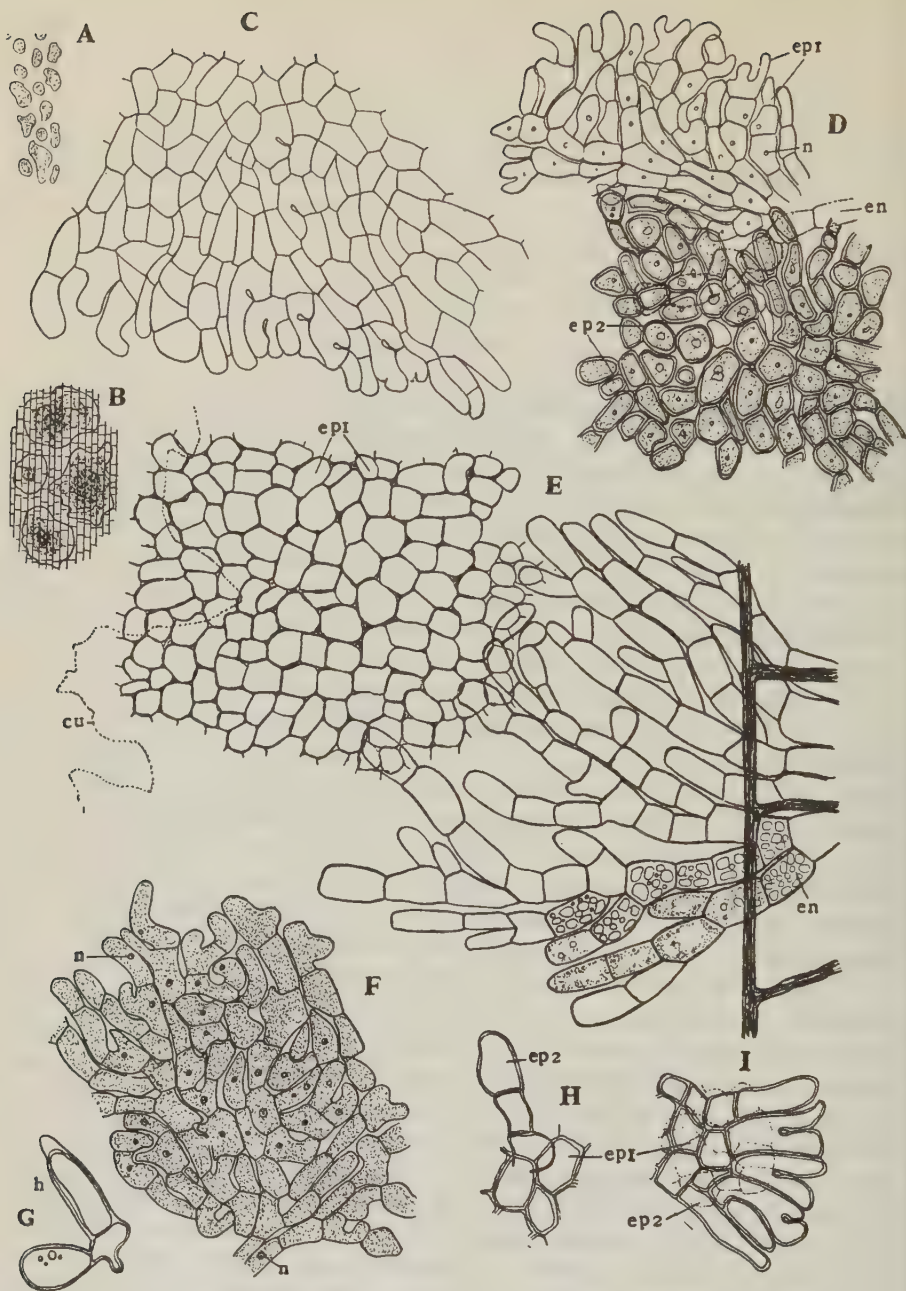


FIG. 1, A-I. *Chrooderma endophytica* n. gen. et sp. A, appearance of the growths on a small part of a bramble shoot. B, four crusts on a larger scale; the dotted areas indicate the regions where there are more than one layer of cells. C, F, marginal parts of two epiphytic strata. D, small part of a crust from above, showing the primary epiphytic stratum (ep 1) and the second layer (ep 2) within the margin; one of the endophytic threads (en) is shown. E, view of part of a crust from the inner surface, showing the epiphytic (ep 1) and a small



an increase to 2 or 3 layers at a varying distance within the margin (Fig. 1, B, D). A series of microtome-sections, for which I am indebted to Dr. N. Carter, displays the same features (cf. Fig. 2, H) and provides further information as to the mode of origin of the several-layered condition.

This results (Fig. 2, I, J, O) from the outgrowth of certain cells of the primary basal stratum (*ep* 1) into short threads (*ep* 2), which for the most part spread horizontally over the surface of the form. An examination of relatively young growths from the surface (Figs. 1, H; 2, D) displays such outgrowing threads (*ep* 2) arising often in considerable numbers from the central (older) part of the basal stratum (*ep* 1), while isolated 1-2-celled upgrowths are commonly to be seen also in the marginal portions. Many of the threads consist of only two or three cells; others are branched, often rather irregularly (Fig. 2, B, *ep* 2), and can be followed up for a considerable distance. Many of the shorter, apparently unbranched, threads, however, stand off, obliquely (cf. Fig. 3, K), or even vertically. Where such short threads are produced in large numbers, as generally occurs in the older parts of the crusts, they are fitted in between the horizontally growing ones and their branches to form a compact aggregate, in which it is exceedingly difficult to trace the individual threads for any distance (Fig. 1, D, *ep* 2). This compact second layer almost completely obscures the underlying basal stratum, which is only exposed at occasional interspaces. The aggregation is not, however, always as dense as this.

Certain branches of the horizontally running threads project vertically and, together with the upper cells of the oblique or vertical outgrowths from the basal stratum may constitute a third layer, which as a general rule does not become as dense as the second (Fig. 2, E). It is possible that in places even a fourth layer may develop in this way. The formation of additional layers in general seems to advance centrifugally and occasionally (Fig. 1, I) extends almost to the edge of the basal stratum. The cells composing the second and later layers tend to be rounded or angular and for the most part possess thick stratified walls.

When the top of a 2- or 3-layered crust is brought into focus one thus sees separate cells, short lengths of 2 or 3 cells running more or less horizontally (Fig. 2, E), and occasional longer threads which can be followed up for a considerable distance. The surface is, however, never altogether level. Since the threads arising from the basal layer may spread in any direction, transverse sections through older crusts in general afford an irregular picture (Fig. 2, C, H). Such sections will only rarely pass through the point of origin of the outgrowing threads or happen to coincide with the direction pursued

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part of the endophytic (*en*) system; the edge of the torn cuticle (*cu*) is seen on the left, a few of the parenchyma cells of the host on the right. G, edge of an epiphytic stratum with a hair (*h*). H, outgrowth of thread (*ep* 2) from cell of basal stratum (*ep* 1). I, edge of basal stratum, with second epiphytic layer (dotted) almost coextensive with it. n, probable nuclei. (A  $\times$  about 4; B  $\times$  about 16; the rest  $\times$  450.)

by the latter (Fig. 2, I, J, O). For the most part the cells composing these threads will be cut through transversely or obliquely so that the crusts appear to consist of a pseudoparenchyma of polygonal cells fitting compactly together. There is in general little evidence that the cells of successive layers, as seen in transverse sections of the crusts, correspond with one another in the vertical direction. The structure is therefore, in part at least, rather different from that of a *Pseudopringsheimia* or *Hildenbrandia*, where the several-layered condition results from upgrowth of straight coalescent threads from the cells of the primary stratum. The method of development of the crusts of the alga under discussion is peculiar in the varied behaviour of the upgrowing threads. Occasional outgrowths from the basal epiphytic stratum, especially near its edge, take the form of blunt unicellular hairs (Fig. 1, G, h).

The intramarginal part of the basal system forms a compact layer of rather small and commonly polygonal isodiametric cells (Fig. 1, C, E, *ep* 1), which often appear arranged in rather indistinct radiating rows. In places the cells are more elongate ( $6.5-7 \mu \times 11-15 \mu$ ) and the filamentous construction of the whole more patent (Fig. 1, D, *ep* 1). Near the growing margin the cells are for the most part markedly elongate in the radial direction ( $6.5-7 \mu$  broad;  $10.5-14 \mu$  long) and commonly prominently lobed (Fig. 1, C, D, I). There are here often evident interspaces between the adjacent threads (Fig. 1, F), and occasional cells may slightly overlap one another. The lobes are, of course, incipient branches, and sooner or later one or both are cut off from the parent cell by periclinal walls. This is much as described by Millardet for *Phycopeltis*, although the lobing is not as regular as in his figures. One may agree with Moebius (1888, p. 230) that such branching is best regarded as monopodial. Occasional lobed cells are to be found well within the edge of the disc (Fig. 1, C). In many places the growing margin has a very irregular contour, although at others for short distances approximating to the segment of a circle. The few young stages found (Fig. 3, A-C) consist almost entirely of lobed cells. The younger cells of the basal stratum have rather thin walls in which a firm peripheral and an apparently less firm inner layer can be distinguished; in the older ones the walls are thicker. The cells are in general comparatively free from granular inclusions, although occasionally containing a limited number of small rounded granules of unknown nature. The cells are in direct contact with the cuticle of the bramble shoot and in transverse section commonly, but by no means always, appear higher than broad (Fig. 2, C, H, *ep* 1).

The cells of the second and third epiphytic strata are for the most part rather larger (diam.  $11-14 \mu$ ) and have denser contents than those of the basal layer. They are commonly full of granular matter and often contain one or more conspicuous rounded bodies which I take to be oil-globules (Fig. 1, D, *ep* 2; 3, L, o), though they do not stain very deeply with Sharlack Red or Sudan III. The appearance of the cells is quite distinct from those of the basal layer and the two are for the most part readily distinguished.

The walls are nearly always thicker and often show evident stratification; in the older growths the cells of the upper epiphytic layers have markedly thickened lamellose walls.

The endophytic system largely occupies a subcuticular position, although certain of its threads are found below the layer of hypodermal parenchyma cells. There is no evidence that they penetrate deeper than this. Over considerable areas the endophytic system is one-layered (Fig. 1, E; 2, L, *en*) and then always shows a very evident filamentous structure. The filaments differ rather markedly in appearance from any of the epiphytic strata. They consist of rectangular or barrel-shaped cells, larger than those of the basal epiphytic stratum, up to twice as long as broad (diam. 8–8.5  $\mu$ ), and exhibit copious monopodial branching. The secondary laterals in places grow backwards or obliquely backwards, more or less parallel with the main filament and, when this occurs, an appearance suggesting anastomosis is sometimes obtained. I have, however, found no evidence of the occurrence of actual anastomoses. Evection is also frequent. In places the threads and their branches are in close contact so as almost to form a continuous stratum; elsewhere the aggregation may be looser. Occasional threads are often seen running longitudinally along the line of demarcation of the files of parenchyma cells of the host; such threads sometimes show numerous short branches, all directed to one side. At certain points the endophytic system is several-layered, some of its branches evidently running beneath the main subcuticular system. The cells are sometimes crowded with irregular opaque whitish masses of various sizes (cf. Fig. 1, E) which are probably of the nature of fat.

The epi- and endophytic systems are connected by way of rounded apertures in the epidermis, which I take to represent places where small prickles have become detached. These apertures (Fig. 2, L) are surrounded by elongate curved epidermal cells (*e*) and are bounded by a thick cuticular ring (*cu*). In areas covered by the epiphytic system of the alga they are largely occupied by its cells (*p*), between which a considerable amount of disorganized cellular material, apparently belonging to the host, is to be found. The algal threads filling the apertures are particularly conspicuous in material stained with cotton blue in lactophenol. Endophytic filaments (*en*) are always found in considerable numbers around such apertures, although it has not proved possible, in tangential sections viewed from the inner surface, to establish a direct connexion between the cells occupying the apertures and the endophytic system. Transverse sections (Fig. 2, C), however, make the relation of the one with the other perfectly clear. When viewed from the outside the threads of the basal epiphytic stratum can be seen dipping into the apertures. Beneath the latter the endophytic system is often several-layered (Fig. 2, C, *en*) and it probably becomes one-layered as it spreads out from these points. In some instances at least it extends further than the epiphytic system. When the latter is not too dense, the



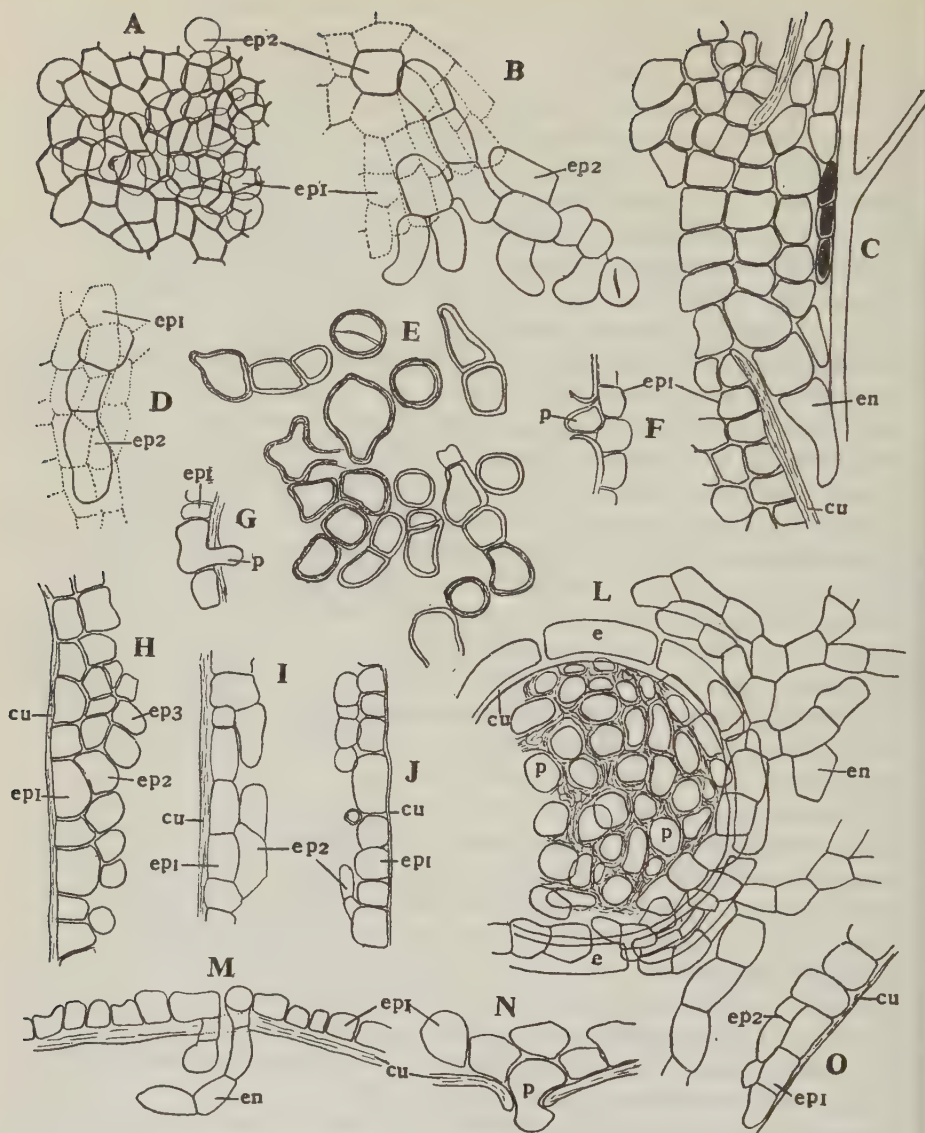


FIG. 2. A-L. *Chrooderma endophytica* n. gen. et sp. A, central part of an epiphytic growth, with basal (*ep* 1) and overlying cells of a second stratum (*ep* 2). B, D, outgrowth of threads (*ep* 2) from cells of basal stratum (*ep* 1), in B extensively branched. C, transverse section of a crust passing through one of the apertures, showing epiphytic (*ep* 1) and endophytic (*en*) systems. E, surface view of a three-layered crust. F, G, M, N, transverse sections of one-layered epiphytic growths showing various instances of possible penetration of the cuticle (*cu*). H, transverse section of a three-layered epiphytic crust. I, J, O, transverse sections of epiphytic systems, showing outgrowth of horizontal threads (*ep* 2) from cells of basal stratum (*ep* 1). L, view of aperture in epidermis from inner surface, showing penetrating (*p*) and endophytic (*en*) threads. *cu*, cuticle; *e*, epidermal cells of host; *en*, endophytic and *ep*, 1, 2, 3, successive layers of the epiphytic systems; *p*, penetrating threads. ( $\times 640$ .)



endophytic threads are recognizable in surface sections at a low focus beneath the superjacent layers (Fig. 1, D, *en*).

It has not been possible to establish finally whether the endo- or epiphytic system is primary, but the fact that on certain parts of the surface only epiphytic growth can be detected, suggests that the endophytic system arises secondarily. Moreover, the relatively few young epiphytic plants that were found (Fig. 3, A-C) were often situated on parts of the cuticle beneath which no endophytic threads could be recognized. In fact it may be that the endophytic habit is fortuitous and that it is the presence of suitable apertures in the epidermis that results in its development. In the microtome sections the cuticle and epidermis are broken at many points and, although some of these ruptures are possibly caused by the knife, others are probably due to natural causes, since examination of the outer surface of the bramble shoots with a lens shows the presence of small areas in which the epidermis is ruptured. There is also a slight suggestion of direct penetration of the cuticle by threads arising from the epiphytic system. Several instances, such as are illustrated in Fig. 2, F, G, M, and N, were observed, some at least of which suggest direct penetration, although this may be taking place along lines in which the cuticle has just ruptured. Dark-coloured fungal hyphae, which are associated especially with the endophytic, but sometimes also with the epiphytic systems, are perhaps concerned in the production of these breaks. The shallow epidermal cells of the host appear to be largely disorganized where the endophytic threads occur, but apart from this there was no evidence of any disintegrating effect.

Where the endophytic system becomes exposed through the above-mentioned cracks in the epidermis it may produce numerous short outgrowing branches like those forming the second and third strata of the normal epiphytic system. These branches are composed of larger rounded cells with dense contents and thick lamellate walls and, except for their direct origin from the endophytic threads, appear just like those borne on the basal epiphytic stratum.

As regards details of cell-structure not much can be said. The endophytic threads in their younger parts often show clearly the pits in the transverse septa which are characteristic of Trentepohliaceae, although these are rarely visible in the older cells which have more strongly thickened walls. The pits are small and not always in the centre of the septum (Fig. 3, D). Similar pits were occasionally seen in the septa of the basal epiphytic stratum. Small bodies, which appear to be nuclei, are discernible in the younger cells of the basal epiphytic stratum (Fig. 1, D, F, *n*) in material stained with cotton blue or haematoxylin. They are not easily distinguishable from the occasional rounded granules found in these cells. The other cell-contents were difficult to decipher, but one or two cells seemed to contain band-shaped structures which were possibly chloroplasts.

In view of the considerable covering formed on the surface of the host, it

is clear that copious reproduction must have taken place, but it has been impossible to arrive at more than tentative conclusions as to the way in which this is effected. More or less numerous cells of the second and third epiphytic strata are distinguished by their particularly large size (diam.

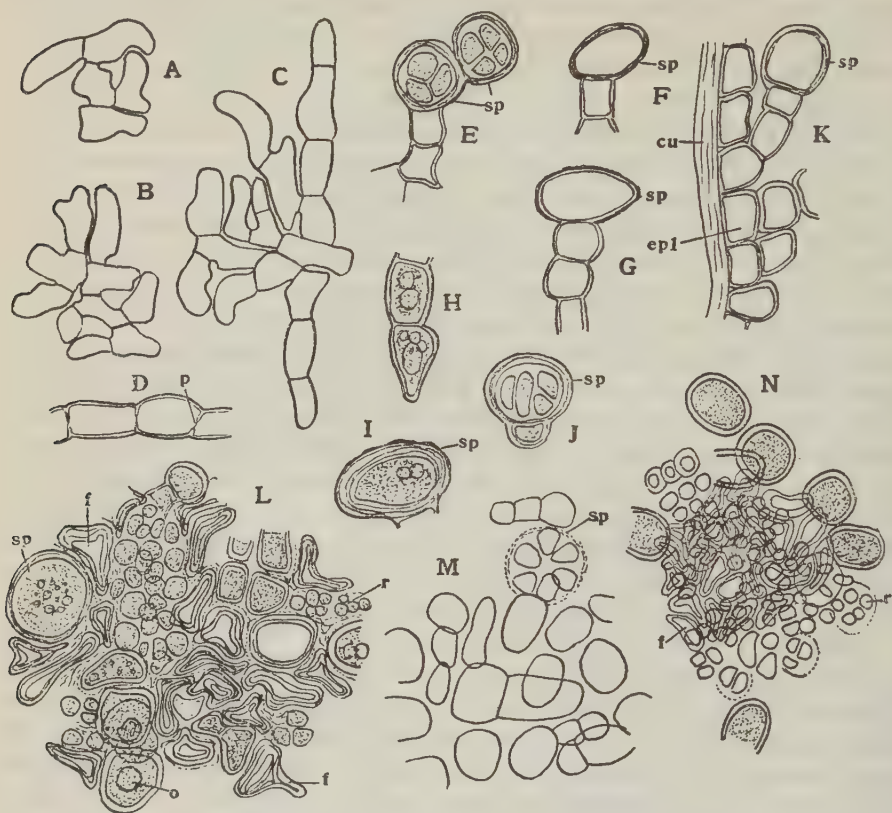


FIG. 3. A-N. *Chrooderma endophytica* n. gen. et sp. A-C, young epiphytic growths. D, a few cells of a young endophytic thread, showing pits (p) in the septa. E-G, I, J, various forms of sporangia (sp). H, thread arising from basal system, from the top of which a sporangium has been detached. K, transverse section of an epiphytic crust with off-standing threads, one terminating in a sporangium (sp). L, upper stratum of a two-layered crust; developing (sp) and collapsed (f) sporangia and reproductive cells (r?). M, ditto, with sporangium (sp) in which the walls are gelatinized. N, ditto, showing empty cells (f) of basal stratum below and possible reproductive cells (r) above. ( $\times 740$ .)

14-18  $\mu$ ); they often contain conspicuous oil-bodies and have more or less thick lamellate walls, the outer layers of which are sometimes partly exfoliated (Fig. 3, E, I, J). Some of these cells are spherical (Fig. 3, L), others are extended perpendicularly to the underlying cells of the threads on which they are borne (Fig. 3, F, G). I suspect that these are in part detachable sporangia which are probably wind-dispersed. A considerable number were indeed found lying loose on the algal crust (Fig. 3, I), but, while this implies

their ready detachment (cf. also Fig. 3, H), it is difficult to say whether it was due to natural causes. A few of these cells, while still *in situ*, showed division of the contents into a number of parts (Fig. 3, E, J), which confirms their sporangial nature and shows that they may form reproductive units also without detachment.

At the same time it is certain that large numbers of cells of the basal and of the second stratum of older crusts may at times be concerned in the formation of reproductive units. The cells in question (Fig. 3, L, N, f) are empty, provided with thick walls, and are usually much compressed and contorted, as if they had collapsed after setting free their contents. Between such empty cells are found others, which are much enlarged (Fig. 3, L, sp) and the increase in size of which is probably responsible for the compression of the former. Within the intact basal epiphytic stratum occasional larger cells with thicker walls are often to be found, and it is probable that these represent immature sporangia. Dehiscence appears to be effected by a split in the wall (Fig. 2, B), although in some instances (Fig. 3, M, sp) the whole membrane appears to become gelatinous. Where the previously described empty cells occur in any considerable numbers, there are nearly always present groups of small, thin-walled, rounded elements of rather unequal size (Fig. 3, L, N, r) which it is tempting to believe are some of the reproductive units liberated from the cells in question. Similar groups of small cells were commonly present at the edges of the crusts, either at the level of the basal or of the second stratum. I am, however, doubtful whether these cells belong to the alga under discussion, since undeterminable colonial green Algae were certainly also present. There can, however, be no doubt that the marginal cells of the basal epiphytic stratum can also liberate reproductive units, since such cells were commonly devoid of contents and their walls provided with a wide aperture. Although it is probable that both types of sporangia liberate motile elements, this can only be established by the examination of fresh material. In *Phycopeltis* the cells of the one-layered epiphytic stratum give rise to swarmers, whilst in the tropical species described by Karsten (1891; cf. also Moebius, 1888, p. 225) others are produced in sporangia borne on threads arising above the general surface.

In certain older growths the basal epiphytic layer appeared to have altogether vanished, so that the irregularly distributed threads forming the superjacent layers seemed to arise direct from the cuticle. It is possible that in this way these threads become free and are wind-distributed as a whole. Fragmentation of the endophytic system into shorter lengths, often composed of cells of rather irregular shape, was also observed.

A number of species of *Phycopeltis* have been described since the genus was established (Printz, 1927, p. 222), but all of these are pure epiphytes with a one-layered stratum. The production of erect or oblique-growing branches terminating in zoosporangia is recorded for several of the tropical species, but it would seem that such branches are always spatially separated



and never formed in the numbers characteristic of the alga described in this paper. Moreover, the branches originating from the primary epiphytic stratum in the latter become, as a general rule, closely fitted together to form an irregular pseudoparenchyma of several layers, and this system is no doubt at first sterile before the end-cells become converted into sporangia. It is this multi-layered condition of the epiphytic growth that most sharply distinguishes the British alga from the known species of *Phycopeltis*, and this alone warrants the establishment of a distinct genus, for which I propose the name *Chrooderma*. A further characteristic of the new genus lies in the production of an endophytic system of threads. As already mentioned, it is possible that the production of such threads is merely the result of the presence of apertures in the epidermis suitable for the penetration of the alga and that it is not an obligate feature. It is probable that a considerable part of the underlying tissue was still living at the time when penetration took place. The narrow threads possessed by several of the tropical species of *Phycopeltis* would presumably permit a penetration through the stomatal apertures of the leaves on which these forms grow, but such a habit has never been recorded.

*Chrooderma* may be regarded as a *Phycopeltis* in which the capacity for the copious production of branches is not limited to the plane in which the primary epiphytic system is extended, but can take place both in the inward and outward directions. The development of numerous outgrowing branches leads to the formation of the several-layered crust, while the ingrowing ones give rise to the endophytic system. There is also a slight resemblance to *Cephaleuros*, although there is no evidence that *Chrooderma* is a parasite. On the other hand, the alga can be interpreted as a condensed *Trentepohlia* of the type referred by Hariot (1890, p. 50; Schmidle, 1897, p. 319) to the subgenus *Heterothallus* which includes only leaf-inhabiting forms. Here the primary filaments (basal system) branch in a single plane and may form an orbicular disc, which in *T. depressa* closely resembles that of a *Phycopeltis*, although the erect system consists of separate little branched threads like those of other *Trentepohlias*.

Reference has already been made to the presence of dark-coloured fungal hyphae in the neighbourhood of many of the threads, especially of those of the endophytic system, although some of the growths were practically devoid of such infection. In no instance did the fungal threads closely envelop those of the alga in the manner characteristic of the lichen *Coenogonium* (Glück, 1896; Skuja and Ore, 1933) or of *Opegrapha filicina* (Bornet, 1873, p. 63) where the algal partner appears to be a *Phycopeltis*. In the material here described the association between algal threads and fungus was always loose and indefinite. On the surface of the bramble shoots were occasional small, rounded, dark-coloured fruit-bodies associated with the hyphae previously mentioned. I am indebted to Prof. Brooks for the opinion that these are probably pycnidia of a species of *Phyllosticta*.



## SUMMARY

*Chrooderma endophytica* n. gen. et sp. is a member of Trentepohliaceae distinguished by the formation of several-layered epiphytic crusts, with which an often extensive growth of endophytic (largely subcuticular) threads is associated, the two systems being connected by threads which extend through apertures in the epidermis of the bramble shoots on which the alga occurs. The basal epiphytic stratum is a continuous layer and shows some resemblance with the disc of a Phycopeltis, while the superjacent layers are formed by outgrowth, from the older cells of the basal system, of threads of varied length which constitute a more or less compact, but irregular, pseudo-parenchyma of larger, thicker-walled cells. Many of the end-cells of the threads become converted into probable sporangia which are readily detached and possibly wind-distributed, while extensive production of reproductive units from the cells of the first and second epiphytic strata also takes place.

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# Wonnacottia, a new Bennettitalean Microsporophyll

BY

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With four Figures in the Text

**T**HIS paper describes a new species of fossil microsporophyll from the Gristhorpe Plant Bed, Yorkshire. Its age is Middle Estuarine (Bajocian), Lower Oolite; and the material was collected in 1938 by Mr. F. M. Wonnacott and deposited in the Geological Collection of the British Museum.

The Type-specimen, V25851, as collected, was an obscure and unattractive compression. Its features were hard to understand because the plane of cleavage had passed through it irregularly and much of the surface was hidden by overlying rock. However, it was greatly improved when some of the rock matrix was dissected away and the drawings shown in Fig. 1 B, 1 C were made from the part and counterpart. Both were then transferred, revealing two more surfaces (Fig. 1 A, 1 D), and comparison of the four aspects reveals the major features of form, while for microscopic details fragments were detached and treated further. It was then shown that the specimen is a microsporophyll and that it belongs to the Bennettitales where it makes considerable addition to their comparative morphology. The other specimens are some small fragments.

## *Diagnosis of Wonnacottia gen. nov.*

Microsporophyll consisting of a simple rachis and a lamina bearing pollen sacs. Lamina as a whole lanceolate, but divided into segments traversed by simple or forked veins, segments bearing on the under side prominent round pollen sacs borne singly or in groups. Substance of the lamina thin. Cuticle well developed; upper without stomata, showing sinuous walled cells; lower with numerous stomata. One subsidiary cell opposite each guard cell. Guard cells showing well-developed crescent-shaped thickenings. Pollen grains oval with a longitudinal groove.

The genus is named after its collector.

## *Diagnosis of Wonnacottia crispa sp. nov.*

Microsporophyll about 12 cm. long, total width of lamina about 1.5 cm., segments of lamina about as long as broad, often becoming narrowed towards the outer margin; often strongly incurved, the outer margin usually being

bent back towards the rachis and concealing the upper surface. Veins visible as ridges on both surfaces, branched once or else unbranched. Sporangia typically 1 mm. in diameter, densely covered with short fine hairs. Pollen grains oval, smooth, typically 30  $\mu$  long, 20  $\mu$  broad.

Upper cuticle of lamina showing epidermal cells with strongly developed jagged thickenings on their lateral walls. Lower cuticle showing epidermal cells with sinuous walls; subsidiary cells small, unthickened. Guard cells flush with the surface, the lines of contact of guard and subsidiary cells wide apart in the region of the aperture. No cuticular papillae present on any epidermal cells, but short hairs with uni- or bicellular bases frequent on the lower side, rare on the upper side. Type-specimen, V25851.

The specific name refers to the curling of the lamina.

#### DESCRIPTION

##### *Gross form.*

The following description is merely intended to supplement and explain the figures.

The leaf base is very slightly expanded and the scar of attachment is terminal. Just above this the rachis is 2 mm. wide (its margins are hidden by rock in 1 B) and it gradually tapers to 0.7 mm. at the highest point preserved, 8 cm. above the base. From this the total length was estimated to be about 12 cm. In the lower part the rachis shows one or two faint longitudinal ridges and also inconspicuous transverse wrinkles probably caused by compression. It is smooth in the part where the lamina is developed. It bears the lamina just above its lateral margins; thus in the region of segments 17, 18 the midrib was found on maceration to have a cuticle 1.0 mm. wide on the upper side above the lamina but 1.4 mm. wide below. These figures indicate attachment of the lamina about midway between the side and top of the rachis.

The rachis contains scalariform tracheides, traces of whose walls can be obtained by incomplete maceration.

The substance of the lamina is thin enough to be slightly translucent. The segments of the lamina are opposite, but it is doubtful whether this is an important character. The four lowest segments are minute and consist of little more than pollen sacs; but the upper segments show progressive enlargement of the lamina up to the level of No. 18. The segments are narrowed as they leave the rachis, and this is often accentuated by the lateral margins being curved over again (Fig. 3 G, 1). The veins are moderately distinct where there are no pollen sacs, and mostly unbranched. The pollen sacs are placed between veins which diverge round them, the larger sacs conceal two or three veins on the lower side.

The pollen sacs are rounded bodies bulging strongly on the lower surface and some of them to a less extent on the upper. Most are borne singly, but some form indefinite groups and occasionally such a group is raised by a short



projection from the lower surface. They are somewhat flattened in preservation and the folds in their walls suggest that they were originally rounded pouches projecting on the under side from a more or less constricted point of attachment; while above they merely form a small hemispherical pimple. The walls of the sporangium are both thicker in cuticle and in underlying tissue than the lamina and are only slowly cleared by maceration. The wall substance reveals only traces of its cellular tissue by maceration. It is lined by an exceedingly delicate cuticle which seems to form a collapsed sac about a quarter as wide as the sporangium. Its walls show faint indications of straight cell walls and in addition small round platelets which appear to consist of solidified oil. These inner cuticles are remarkably free from pollen in most of those examined, but in one sporangium of the type-specimen about a hundred grains are still present.

The opening of the pollen sacs has not been fully observed. Before maceration some of the pollen sacs show a deep depression which might be the mouth of the sac, but might also be a fold caused in crushing. After maceration it is sometimes possible to find a long crack or an elliptical hole in the outer cuticle of the sporangium from which cuticle projects inwards and is probably connected with the cuticle lining the pollen sac. This feature was found on the lower side; there is never any sign of a break on the upper side. It may be anticipated that the study of additional material by other methods would yield good results.

### *Cuticle.*

1. *Lamina.* Both cuticles are of medium thickness (about  $2\ \mu$ ) and fairly tenacious. The upper (Fig. 2 G) consists of fairly uniform cells, tending to be rectangular along the veins, less regular between them. The veins are not otherwise indicated. The cell walls are sinuous and have strongly jagged thickenings (Fig. 2 E). Trichomes are rare and stomata absent.

The lower cuticle (Fig. 2 H) has numerous stomata, the concentration being lowest near the rachis, highest near the distal margin where there are about 100 per sq. mm. The stomata are generally scattered but less frequent over the veins, while trichomes which are also scattered are more numerous over the veins (Fig. 2 F). The lateral walls of the epidermal cells appear thinner than on the upper side, and more sinuous, but with less developed jagged thickenings. The surface of the cells like those of the upper side is flat and appears finely and evenly granular. The stomata are irregularly orientated. The subsidiary cells are small but usually extend beyond the lateral plates of the guard cells; their outer walls are very distinct and not very sinuous and their surface is little if at all thicker than that of other cells. The guard cells show a broad thickly cutinized area round the aperture, which seems to be flush with the general surface and rather extensive crescent-shaped lateral plates extending obliquely inwards along the common wall of

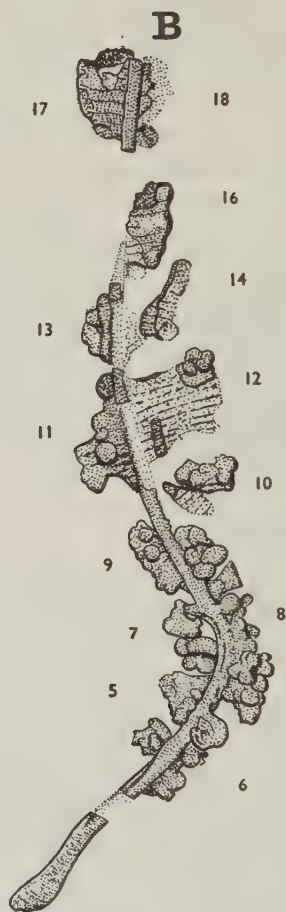
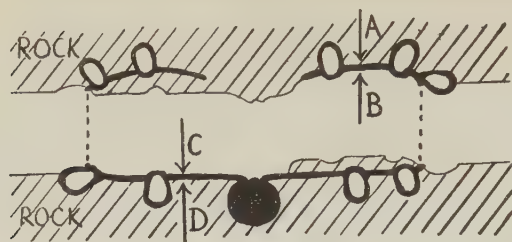


FIG. 1, A and B. The diagram at the top relates the four aspects represented respectively in A, B, C, and D. The plane of cleavage changes, this corresponds to the cleavage at the level of segment 12. A, transfer of 'part', V25851A; B, 'part' as exposed on rock surface. Both figures  $\times 2$ .

the guard cell and subsidiary cell. These lateral plates are sometimes as long as the subsidiary cells, and although well defined are made of a rather thin cutin layer. The cuticle of the stomatal aperture does not extend far inwards. The lines of contact of the subsidiary cells and guard cells (where the lateral

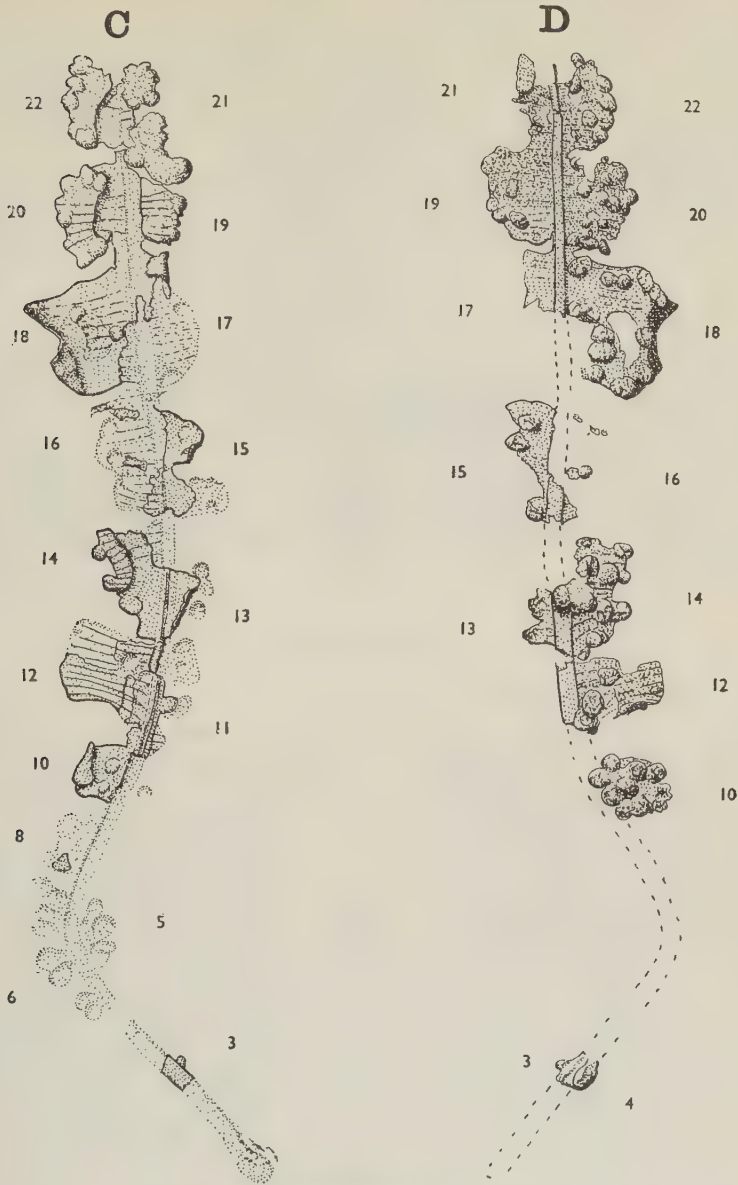


FIG. 1, C and D. C counterpart as originally exposed; D transfer of counterpart (V25851B). Both figures  $\times 2$ .

plates meet the surface) are characteristic; they diverge as they approach the aperture from the poles and then run parallel or continue to diverge slightly; very rarely do they converge on the aperture.

Trichomes are rather frequent. They consist of a thickened base of one,

or more commonly two, thickened and somewhat raised cells bearing a short, thinly cutinized hair which is usually lost in preparation (Fig. 2 A, B).

2. *Rachis*. The rachis and midrib have a thicker cuticle showing large rectangular cells, those on the upper side with distinct jagged thickenings, but those on the lower side with straight walls. Trichomes are frequent and of rather large size (Fig. 2 C), but stomata absent.

3. *Sporangia*. On approaching the sporangia, both upper and lower cuticles of the lamina are modified in a similar way. The cells all become polygonal and isodiametric, their walls straight, without jagged thickenings (Fig. 3 H). Stomata become rare, but trichomes are extraordinarily common and on the sporangia themselves nearly every other cell bears them. The cuticle of the sporangia is much thicker than that of the lamina, but owing to the effects of folding and crushing, it could not be studied very fully and it is not known how it is modified in different parts of the sporangium.

4. *Pollen*. The pollen grains have a well-developed cutinized extine which is smooth and has a deep longitudinal groove. Those figured were inside a pollen sac which though open had still about a hundred grains on its inner wall; a good many of the same type are scattered over the epidermis of the lamina.

## DISCUSSION

### 1. *Inclusion in the Bennettitales*.

The interest of *Wonnacottia* depends not on its own organization but on comparison with other Bennettitales. Its classification in this group is based on evidence given by the structure of its epidermis.

The whole aspect of the cuticle including its sinuous cell walls and the peculiar cutinized thickenings of the guard cells, is typically Bennettitalean. The most important single point is given by the subsidiary cells. These in the Bennettitales are always two in number, placed opposite the guard cells, the whole row of four cells having evidently been formed by the division of a single surface cell of the embryonic leaf (syndetocheilic origin). This character was pointed out by Thomas and Bancroft (1913) and has been developed by Florin (1931, p. 507, 1933a, 1933b). In the other 'Cycadophytes' (the name given to the assemblage of Cycads, Pteridosperms, and various other Mesozoic fossils of more or less Cycad-like aspect) the stomata are different, being of the usual gymnosperm type. The subsidiary cells are more than two, and by no means exactly opposite the guard-cells, evidently having been formed from different surface cells in the embryonic leaf.

This character has been much used to divide the fossil Cycadophytes into two clear-cut groups. It is easily demonstrated and is characteristic of the stomata from the whole plant, not merely of the leaves. It has been tested as rigorously as possible by Florin, who examined the stomata of much living or fossil material of known classification without finding any exception.



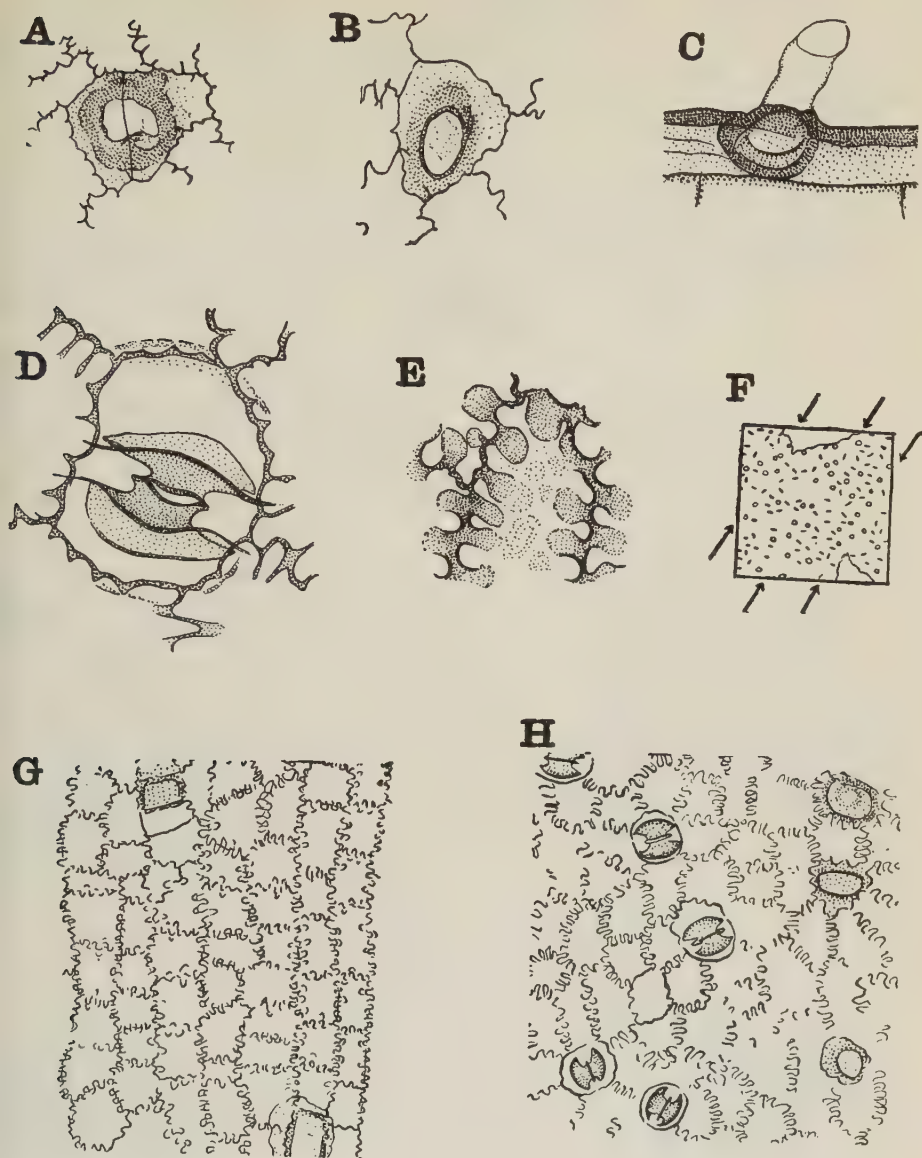


FIG. 2. A-H. A, B, hair bases on under side of lamina (slide H),  $\times 400$ . C, hair base and part of hair on midrib (slide C),  $\times 250$ . D, typical stoma from lamina (slide H),  $\times 800$ . E, details of cell walls of upper epidermis of lamina (slide G),  $\times 800$ . F, portion of lower epidermis, near the distal margin, showing the position of veins (arrows), hair bases (circles), stomatal apertures (short black lines). The square is 1 sq. mm. (slide H). G, upper epidermis (slide G),  $\times 250$ . H, lower epidermis (slide H),  $\times 250$ . All from V25851, type-specimen.

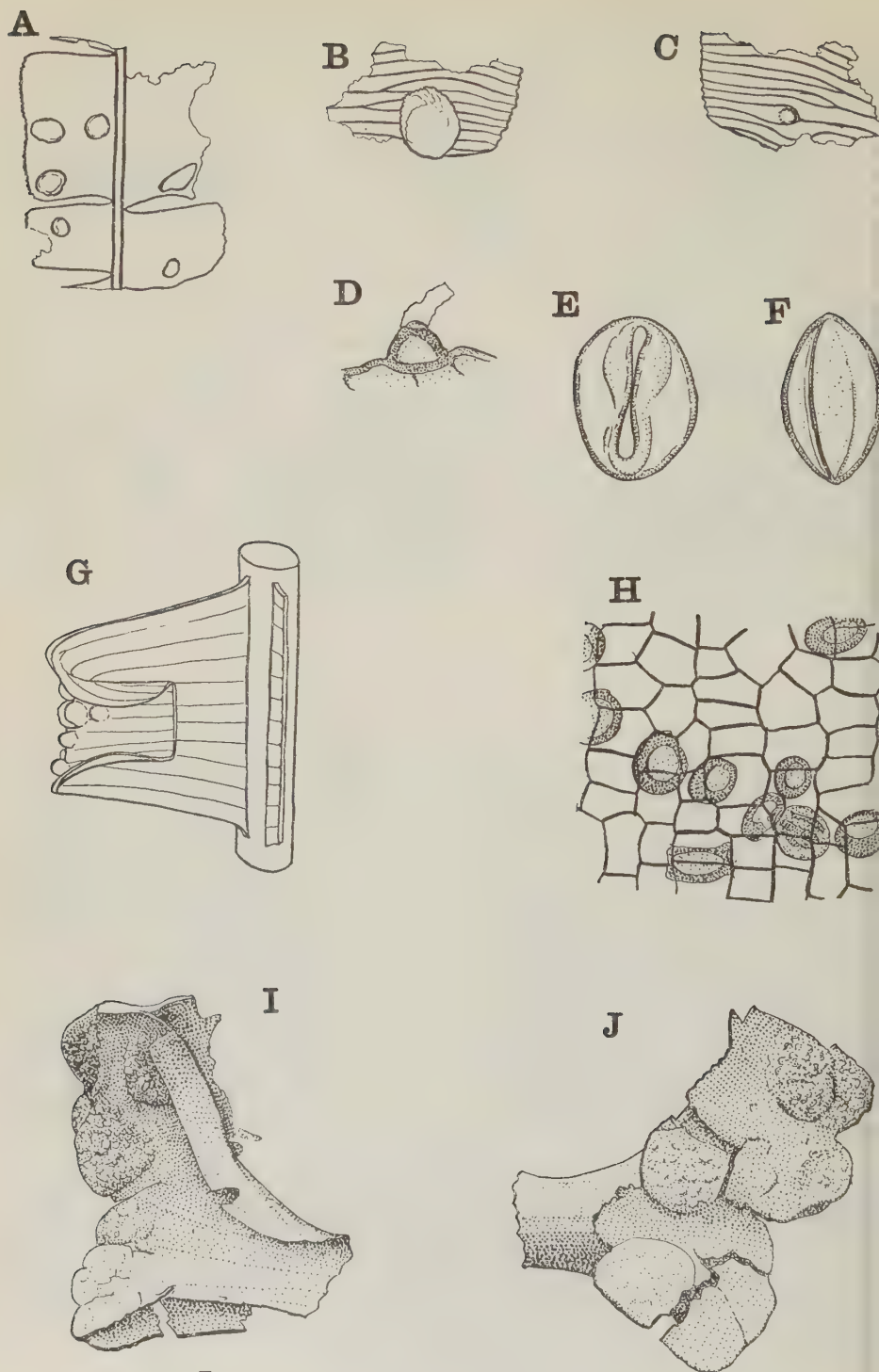


FIG. 3, A-J. (For description see opposite page.)

Its use in classifying the numerous isolated Mesozoic 'Cycadophyte' organs into Bennettitales and other 'Cycadophytes' has enormously cleared their taxonomy, and no hint of conflict between this and other evidence has yet emerged. This character is thus one already proved to have wide application and there is no sign of limit to its use among these plants. As it happens, *Wonnacottia* shows other Bennettitalean features in its cuticle, as well as characters like the pollen-grain shape and the scalariform thickening of the xylem which though not diagnostic are fully consistent with this position. In no respect does it approach closely any other group.

Its position as an organ of a member of the Bennettitales is therefore considered to be secure.

## 2. *The parent plant of Wonnacottia.*

It is likely enough that a plant would produce more leaves than microsporophylls (of similar size). The leaf should therefore be a commoner fossil. In this well-known locality, whose leaves have been assiduously collected for a long time, we might expect the leaf to be a species that has already been described.

There is in fact strong evidence pointing to a particular leaf, namely *Anomozamites nilssoni* (Phillips) Seward. Such isolated organs as these can be linked by evidence of various types.

There is evidence of association, which can be very valuable under some circumstances, though it is merely suggestive in this case. The small piece of rock with the type specimen shows no leaf to which *Wonnacottia* could belong, but another (Fig. 3 A-C) is packed with fragments of *A. nilssoni* and of the scale leaf of *A. nilssoni* which has not hitherto been described. Two other pieces of rock showing *A. nilssoni* yielded fragments of *Wonnacottia* when macerated in bulk; again there was no other associated leaf to which this microsporophyll could belong.

There is full and perfect agreement between *Wonnacottia crispa* and *A. nilssoni* in the structure of their cuticles which are known from excellent material of both midrib and lamina. The two are in fact indistinguishable in thickness, shape, and arrangement of epidermal cells, details of cell walls, stomata, and hairs. No other leaf is known which agrees so closely with *Wonnacottia*, though *Nilssoniopteris vittata* is like it in some respects. That leaf differs, however, in its stomata, the subsidiary cells often produce projections over the guard-cell surface and their inner margins nearly always

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FIG. 3, A-J. A, underside of second specimen in transfer showing the occasional, large sporangia;  $\times 2$ . B and C, two fragments isolated from the lower part of A in preparation. B, underside showing the large sporangial swelling; C, from above, showing the minute sporangial projection between two veins;  $\times 5$ . A, B, C are V21394. D, hair base in side view of sporangium wall (slide E),  $\times 250$ . E, F, pollen grains (slide C),  $\times 800$ . G, restoration of a leaf segment seen from above, to make clear the nature of I. H, upper epidermis, over a sporangium. I, J, portion of leaf segment cleaned with HF; I, from beneath; J (as C) from above. I, J  $\times 10$ . D-G represent the type-specimen.

converge on the stomatal aperture. The microsporophyll of *N. vittata* is already known (*Williamsoniella*).

Evidence can also be provided by specimens of intermediate character. The second specimen (V21394) differs in being much less fertile, having one to three pollen sacs on each segment. It agrees exactly with typical *A. nilssoni* leaves in the shape of the segments and in their venation and in particular in the characteristic little points in which the veins end. The pollen sacs are most inconspicuous on the upper surface, and it is only when the specimen is transferred and the lower surface is seen that its nature is obvious. Such a specimen might equally well be classified as *Wonnacottia* or as *Anomozamites nilssoni*.

These kinds of evidence show beyond reasonable doubt that the two organs belong to the same plant species.

This identification of the microsporophyll of *A. nilssoni* shows that the leaf-genus *Anomozamites* is partly unnatural. Another species, *A. minor*, has a fructification called *Wielandiella angustifolia* in which the microsporophylls although imperfectly known are differently organized. This might have been suspected on general grounds, the Bennettitalean leaf genera *Anomozamites* and *Pterophyllum*, for example, being distinguished by the not very profound character of the relative length and breadth of their leaf-segments. There is indeed evidence, though not strong enough to exclude doubt, that various different types of fruit may belong to species of *Pterophyllum*. This will cause no practical difficulties; specimens of *Anomozamites* will be dealt with as hitherto. The recognition that this is a 'form-genus' including the similar-shaped leaves belonging to a number of genera of the Bennettitales makes it clear that it is useless to try to characterize *Anomozamites* more precisely as though it were a single natural genus.

### 3. *Special morphology of Wonnacottia.*

The leaf-like texture and the leaf-like cuticle of the lamina of *Wonnacottia* strongly suggest that it was photosynthetic. The curving-over of the surface probably occurred late in life, after the epidermis had matured in the normal position, since no differences in either thickness of cuticle or character of the cells occur where the lamina bends. It will be noticed, too, that some segments are less curved than others, and no curvature occurs in the less fertile specimen.

The leaf-like rachis of *Wonnacottia* shows that it is a 'free' microsporophyll, not an organ united laterally with others to form a cup or disc. The leafy character suggests that it is unlikely to have formed part of a flower-like organ; the well-developed cuticle of *Wonnacottia* stands in contrast with that of flowering plant stamens, and the microsporophylls of Bennettitalean flowers. It may possibly have been a rather long-lived organ borne on a stem in much the same way as an ordinary leaf.

The pollen sacs appear to open on to the lower side and most are free.



Such groups as occur are indefinite, and there is no hint of syngonium formation. Their fine structure is unknown.

#### 4. *Comparative morphology of Wonnacottia.*

The main interest of *Wonnacottia* lies in the fact that it is a leaf-like sporophyll, a thing of great rarity among the later seed plants. It is of particular interest in relation to the other Bennettitales, as it can be used to support the argument that the microsporophylls of this family are primitively leaf-like.

Fully leaf-like sporophylls, comparable with the ordinary fertile leaves of a fern, are only known in seed plants among the carboniferous Pteridosperms, and even here they are not general, as others produced sporophylls which differed from leaves in having no lamina and often, too, in their mode of branching. Partially leaf-like mega- and micro-sporophylls are known in various Mesozoic Pteridosperm derivatives including the Caytoniales, where the branching recalls a fern leaf but there is little or no lamina; in megasporophylls of the Mesozoic Cycad *Palaeocycas integer*, where there is a moderate-sized leafy lamina; and in *Cycas* itself. Pinnate branching of Bennettitalean microsporophylls is discussed below. In no Tertiary or recent seed plant are the microsporophylls like ordinary leaves.

In morphological theory leaf-like sporophylls were once frequently postulated, but this was at the time when seed plants were supposed to be derived from 'ferns' as we know them. For various reasons the Psilophytales have displaced the ferns from this position of ancestors-elect, and it is accordingly supposed that certain ramulus systems became specialized as reproductive organs, others as photosynthetic organs, and no such organ as a leaf with both photosynthetic lamina and sporangia occurred in the history of most seed plants. This view is fully set out by Zimmermann (1930) and briefly outlined with special relation to the carpel by Thomas (1931-9). Undoubtedly the rarity of leafy sporophylls among seed plants has favoured this view.

Some of the most leaf-like microsporophylls of Mesozoic plants are found in the Bennettitalean genus *Cycadeoidea* where they are almost fern-like in their richly pinnate branching, but even here they are specialized reproductive organs without any green lamina. The following discussion deals with the possibilities of evolution of this type of sporophyll, and a case is built up with the help of evidence from *Wonnacottia* that it is modified from a fully leaf-like organ.

We will first consider the way the microsporophylls were produced. *Wonnacottia* is evidently a free microsporophyll; the only other Bennettitalean genus with free microsporophylls is *Williamsoniella*; in the other genera they are united at the base with neighbouring microsporophylls to form a 'disc', while in some *Williamsonia* species the union extends so far that the microsporophylls look a good deal like the corolla tube of a

Convolvulus flower. It is commonly argued that this condition is secondary in the flowering plants, and it would seem likely that it is secondary in the analogous flowers of this family.

It is unlikely that *Wonnacottia* was produced in a structure resembling a flower. Its thickly cutinized petiole suggests an independent and long-lived organ like a leaf. In this it is at present alone, as in all other genera the microsporophylls form a whorl on a highly specialized reproductive shoot or flower. It may not however prove to be unique, for it should be remembered that while complete Bennettitalean flowers which make handsome fossils have been closely studied, small reproductive organs such as free microsporophylls have been neglected to an astonishing extent.

With regard to its branching, *Wonnacottia* occupies an intermediate position (Fig. 4). Simple sporophylls are produced by *Williamsoniella*, most species of *Williamsonia*, and in *Cycadeoidea colossalis*; pinnately compound ones in *Williamsonia spectabilis* and other species of *Cycadeoidea*. If, as is argued below, the spore capsules of *Cycadeoidea* and *Williamsonia* represent leaf segments, the branching is throughout one degree higher than this; the majority of *Cycadeoidea* species then being bipinnate, but the differences remain. The taxonomic treatment of the species with more or less branched sporophylls shows how little significance has been attributed to this difference. Perhaps this is because the ferns have made us familiar with the idea that in organs with a potentiality for pinnate branching the extent to which this branching is carried is taxonomically unimportant.

The hypothesis developed here that in all Bennettitales the microsporangia are produced embedded in the surface of a modified lamina raises the question, on which side of the lamina? Here our knowledge of fact is inadequate to supply the answer.

In *Wonnacottia* probably all opened on to the lower side (this side being determined in relation to epidermal structure and also the level of attachment of the lamina to the midrib). In *Cycadeoidea*, while the evidence is inconclusive, the orientation of the synangia in bud suggests the upper side. In the simple *Williamsonia* sporophylls and in *Williamsoniella* the synangia are adaxially placed on the sporophyll, but if we regard these as leaf segments on the swollen midrib their position is hardly relevant to this question.

Other important questions which we are in no position to discuss are the relation of the sporangia to the veins and the meaning of the somewhat sunken position they always seem to occupy in the lamina.

The spore-bearing appendages are varied in the different genera. Our view of the whole has tended to be based on *Cycadeoidea* which, because of Wieland's work, is the best-known genus. Here they are produced in little two-valved capsules which look like the synangia of *Marattia*. Wieland (1901, 1906) seems to have held both views of their morphological nature, considering them sometimes as *Marattiaceous* synangia; sometimes as folded pinnules with superficial sporangia. He does not make a clear contrast

between these views which he may have felt to be compatible. In the Marattiales, however, the numerous living and fossil genera indicate clearly that the Marattia synangium is of the same nature as the sorus of more or less

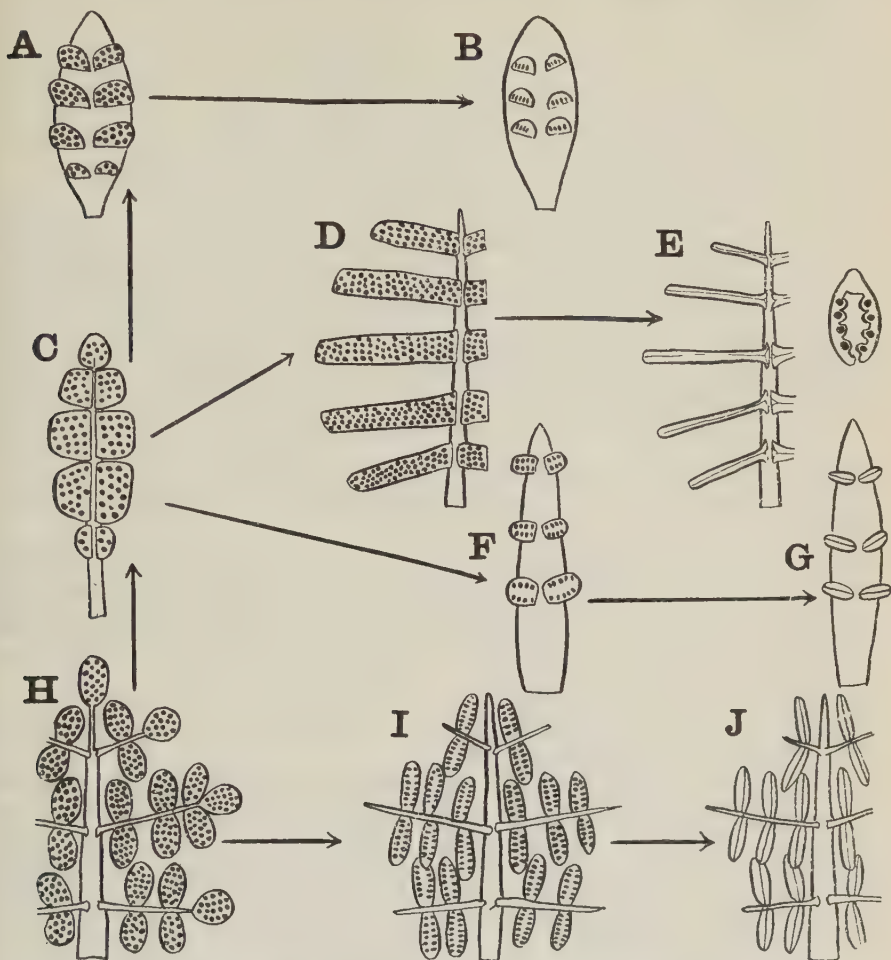


FIG. 4, A-J. Diagram to relate the gross forms of the Bennettitalean microsporophylls. All simplified. A, *Bennettistemon amblum* (sporangia on erect plates on broad midrib). B, *Williamsoniella*. C, *Wonnacottia*. D, intermediate leading to E, *Cycadocephalus* (one spore capsule shown in section). F, intermediate leading to G, *Williamsonia*. H, *Bennettistemon ovatum* (appendages), branching hypothetical. I, intermediate leading to J, *Cycadeoidea*.

free sporangia of Angiopteris, no reduced lamina being involved. There is therefore a real difference between the views. Of other writers, most have simply described the spore capsules of Cycadeoidea as being like Marattia synangia, others have mentioned the pinnule hypothesis but without approval (Scott, 1923; Chamberlain, 1935; Kräusel in Engler, 1926). Zimmermann (1930) develops the view that they are no more than 'telome stands', clusters



of fertile ultimate ramuli, the question whether they constitute pinnules being meaningless. This would be consistent with their close comparison with *Marattia* syngangia.

Spore capsules of the Cycadeoidea type have been shown to occur in the Yorkshire Jurassic species of *Williamsonia* (Nathorst, 1911), though there is no evidence that they were produced by all species referred to that comprehensive genus. Very similar isolated spore capsules have been described as *Bennettistemon bursigerum* Harris (1935), though here the walls are more massive and probably photosynthetic, differences of some significance.

Another genus with spore capsules is *Cycadocephalus* Nathorst (1911), in which the capsules appear to consist of a slender tube, lined with very numerous sporangia. The whole structure was described as a 'synangium', but in view of the facts that it has a strong vascular bundle, and the epidermis is provided with stomatal bands, Nathorst considered the possibility that it might represent a rolled fertile leaf segment; and subsequent writers have favoured the leaf segment view. Although it may not be impossible, it will be seen to be difficult to imagine the direct change from the Cycadeoidea synangium to the Cycadocephalus type or vice versa (Fig. 4).

It is possible that the little known *Weltrichia* is closely similar to *Cycadocephalus*.

In *Williamsoniella* the spore capsules are different again, each consisting of a single row of sporangia partly immersed in a rather fleshy microsporophyll. This type has been supposed to be reduced from something like the *Williamsonia* type, but on that view a double row of sporangia would have been expected in *Williamsoniella*.

*Wielandiella* may be excluded from discussion, its minute microsporophylls being too imperfectly known.

Three types of microsporophyll are now known in which the sporangia are distributed over a lamina instead of being aggregated into a sort of synangium or capsule. The simplest of these is a little oval scale called *Bennettistemon ovatum* (Harris, 1932), covered on one surface with small round sporangia. The opposite side shows a midrib and the thin cuticle shows stomata of Bennettitalean type; they are not numerous and there is no suggestion that the organ was photosynthetic. It is possible that this scale was a complete sporophyll, but it is equally possible that it was a terminal lobe of a branched organ (Fig. 4).

*Bennettistemon amblum* Harris (1932) is more elaborate. This is a rather larger lanceolate scale bearing on its surface two rows of transversely placed semicircular plates. Each plate carries some twenty or thirty sporangia which seem to open by a split facing the apex of the sporophyll. The opposite side of the lanceolate scale is nearly smooth and shows a thin cuticle with rather numerous stomata. The possibility exists that this organ was photosynthetic. In this species the main lanceolate scale may represent a broad midrib, the small semicircular plates reduced leaf segments.



The third is *Wonnacottia*, described here, by far the most leaf-like in character.

The hypothesis put forward in this paper is that a primitive Bennettitalean microsporophyll was a richly branched, green, leaf-like organ with a well-developed lamina bearing scattered, sunken sporangia over its surface. It was produced as a separate organ, like a leaf. Such organs were liable to be borne in crowded whorls on special reproductive shoots or 'flowers'. Here they were protected till maturity by scale leaves and became reduced accordingly. The lamina lost its photosynthetic power, becoming purely a pollen-forming organ, and being modified in a variety of ways, all readily related to the expanded types (Fig. 4). The development of free branches was liable to be suppressed, and the midribs of the sporophylls were liable to become broad organs protecting the pollen sacs much as petals do in many angiosperms. This protective power was further increased by the union of adjacent microsporophyll bases to give the campanulate structures of certain *Williamsonia* species (Wieland, 1914, 1916). With this specialization of reproductive function, the life of the microsporophyll became shortened, the entire male flower or the microsporophylls being shed soon after the pollen was liberated.

It will be seen that this hypothetical ancestral type has been built up by imagining an organ from which all the known genera could be derived by simple changes. To go further, as for example to a telome+phyllome system, we should have to use evidence derived from plants of problematical relationship with this family.

It may be asked whether geological evidence of relative age provides any support for this supposed direction of evolution: it shows nothing at all. Most of the types are represented at the very beginning of the Jurassic at the stage when our knowledge of Bennettitalean microsporophylls begins. The fact that the Cycadeoidea type outlasted the others is hardly relevant.

This discussion of the microsporophyll serves mainly to exclude some of the too numerous possibilities of evolutionary connexions, and to direct attention to certain questions of fact about microsporophylls, but it does not seem to bring the Bennettitales any closer to other groups. The hypothetical microsporophyll is vaguely Pteridosperm or fern-like, but not closely similar to any known type. It is indeed out of the question that the microsporophylls should relate the Bennettitales to any other family. Though there is impressive agreement in stem structure between some Bennettitales and Cycads, other organs, the stomatal apparatus, and the gynaeceum are separated by morphological gulfs so wide that few care to cross in imagination.

#### SUMMARY

1. *Wonnacottia crispa* gen. et sp. nov. is described, a microsporophyll, from the Middle Jurassic rocks of Yorkshire.
2. It consists of a rachis and a pinnately divided lamina with embedded pollen sacs opening on to the lower surface.

3. *Wonnacottia* is referred to the Bennettitales, and to the same plant as the leaf *Anomozamites nilssoni*.
4. It is concluded that *Wonnacottia* was a green leaf-like organ, produced separately rather than in a flower.
5. An attempt is made to reconstruct a hypothetical microsporophyll from which all known types of Bennettitalean microsporophylls could be derived.

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# Wollea Bharadwajae sp. nov. and its Autecology

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With thirty-one Figures in the Text

## INTRODUCTION

WHILE studying the biological productivity of freshwater jhils, ponds, and pools of northern India the writer came across an interesting blue-green alga whose morphology agreed with the genus *Wollea* Born. et Flah. As the alga exhibited peculiar differences in habit during the course of its life-history it was felt desirable to work out its autecology.

In its initial stage the alga remains attached to the submerged soil along the whole bank of the Botanical Garden pond of the Benares Hindu University, from which it was collected for the first time on May 25, 1939. Since then it has also been found to grow in other ponds of the Benares district as well as in Saruan jhil in the Gorakhpur district. As these localities are far away from the experimental station, the morphological studies described below have been made on living material obtained from the first habitat only; most of the ecological observations have also been carried on there. As the water-level in the pond decreases the alga gradually encroaches upon the littoral zone which may be up to 5 ft. in extent.

## MORPHOLOGY OF THE ALGA

The plant makes its appearance in the form of small club-shaped blue-green vesicles (Figs. 1-3) aggregated to form a large expanse, covering the mud completely in the littoral zone, and recalling the habit of a small *Valonia* (Figs. 1-6). The vesicles of the plant gradually grow in size, assuming ultimately a more or less cylindrical form (Figs. 6-7). The plant body consists of a solid cylinder of mucilage the outer layer of which is firmer than the inner portion in which the trichomes are embedded. The mature colonies are, therefore, composed of numerous unbranched cylindrical sheaths of more or less firm texture, radiating from a single point (Fig. 8). They appear like the fingers of a glove, attached by one end to the mud bank of the pond, and at the other end being free-floating. In the littoral zone, however, the vesicles stand out almost erect from the submerged mud. Each sheath is usually 4-5 cm. long and 2-2.5 mm. broad. It readily takes up all kinds of stains (i.e. aqueous methyl green, alcoholic safranin, methylene blue,

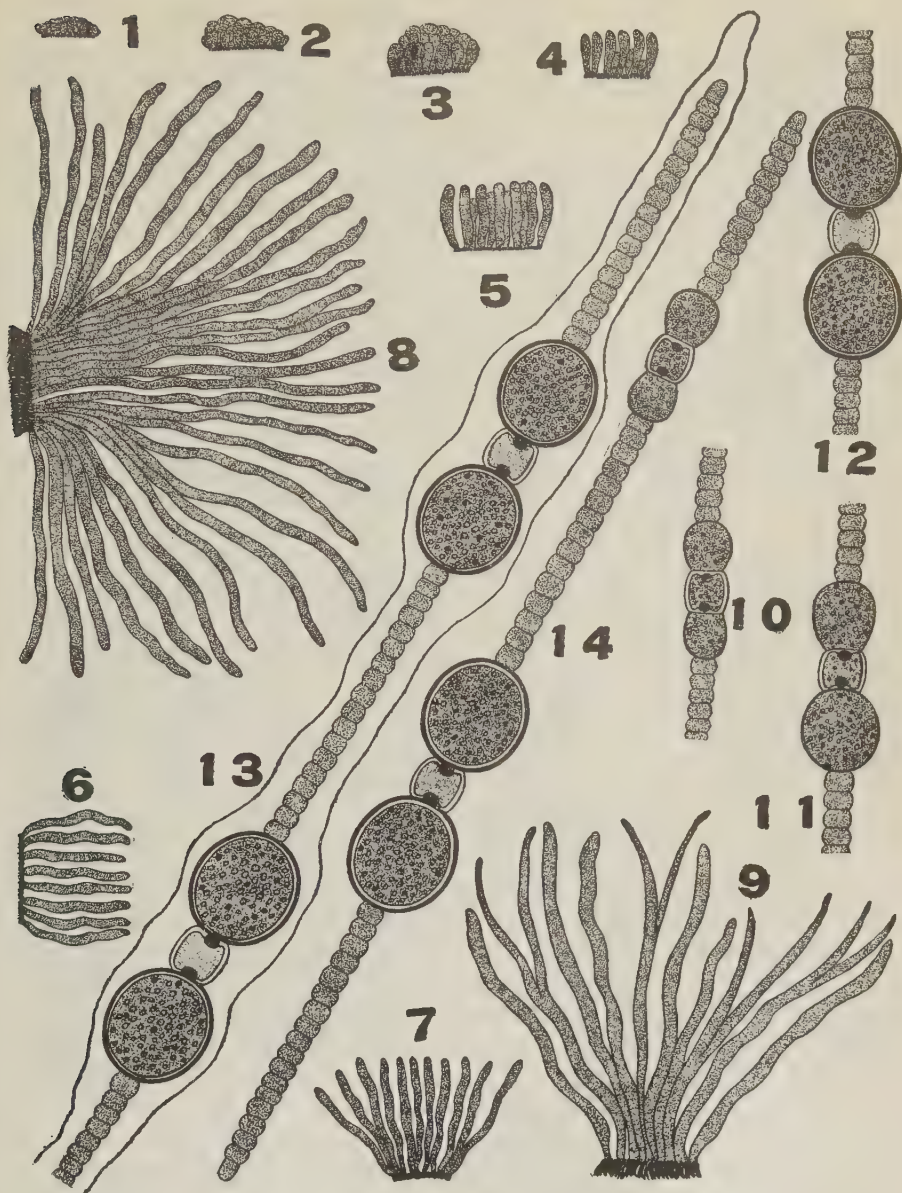
Heidenhain's haematoxyline, &c.). The reactions with aqueous methyl green and alcoholic safranin show an affinity of the sheath for basic aniline dyes, and thus indicate its pectic nature (cf. Bharadwaja, 1933). Chlor-zinc-iodine does not stain the sheath violet even after a long immersion, showing thereby the absence of cellulose in the sheath. A dissection of the sheath under the microscope shows numerous imbedded trichomes; these are generally straight, sometimes curved, and they run almost parallel to each other (Fig. 15). In some cases they overlap, but they never become entangled. They measure up to 1 mm. in length and usually taper at the extreme ends, the conical end-cells being rounded at the apices.

The cells are barrel-shaped, the breadth ( $3.5-4.8\ \mu$ ) being the same or somewhat greater than the length ( $2.5-3.5\ \mu$ ) with a deep constriction at the cross-walls (Figs. 13 and 14). The apical cell measures  $2-3\ \mu \times 4.3-5.8\ \mu$ . The dissepiments are fairly distinct, but they become clearer on treatment with an alcoholic solution of iodine. The cell-contents are deep blue-green and coarsely granular. The walls and the contents of the cells show practically the same reactions to various stains and reagents as have been described by Bharadwaja (1933) for *Cylindrospermum muscicola* var. *kashmirensis*.

The pale blue-green heterocysts, usually up to six or more in number, are distributed at more or less regular intervals throughout the length of the trichome. They are more or less barrel-shaped with flat ends, the contents being at first granular but later homogeneous. They are usually larger than the vegetative cells, being  $5.8-7.0\ \mu$  broad and  $6.0-7.5\ \mu$  long. The observations made in regard to heterocysts by Fritsch (1904) in *Anabaena* and by Bharadwaja (1928) in *Spelaeopogon Kashyapi*, and the reactions of their walls and contents to various stains and reagents described by Bharadwaja (1933) for *Cylindrospermum muscicola* var. *kashmirensis*, *Aulosira Fritschii*, and *A. prolifica* are in close agreement with those of the present alga. The heterocysts retain their pigment till late in the life-history.

The spore-development in the alga begins at the close of the vegetative period, extending from 4 to 6 days, and the procedure is essentially similar to that described by Fritsch (1904) for *Anabaena Azollae* Strasb. The spores are formed singly on either side of a heterocyst (Figs. 13 and 14). The mature spores are yellowish-green, containing numerous large granules of different size, spherical or sub-spherical, occupying the whole protoplast. They have a smooth thick dark-brown exospore and a thin hyaline endospore (Figs. 16-18). When detached from the trichomes their thin polar ends exhibit distinct pores (Figs. 16-18). The spores, therefore, maintain their connexion with the adjoining cells and heterocysts till the former are mature. It has also been observed that the granular appearance of the contents of the heterocysts is invariably lost as the adjoining spore matures and exhibits a granular content (Figs. 10-12). This shows that the heterocysts serve to supply nutritive material to the spores. Further support for this view is given by the fact that when, on the onset of unfavourable conditions



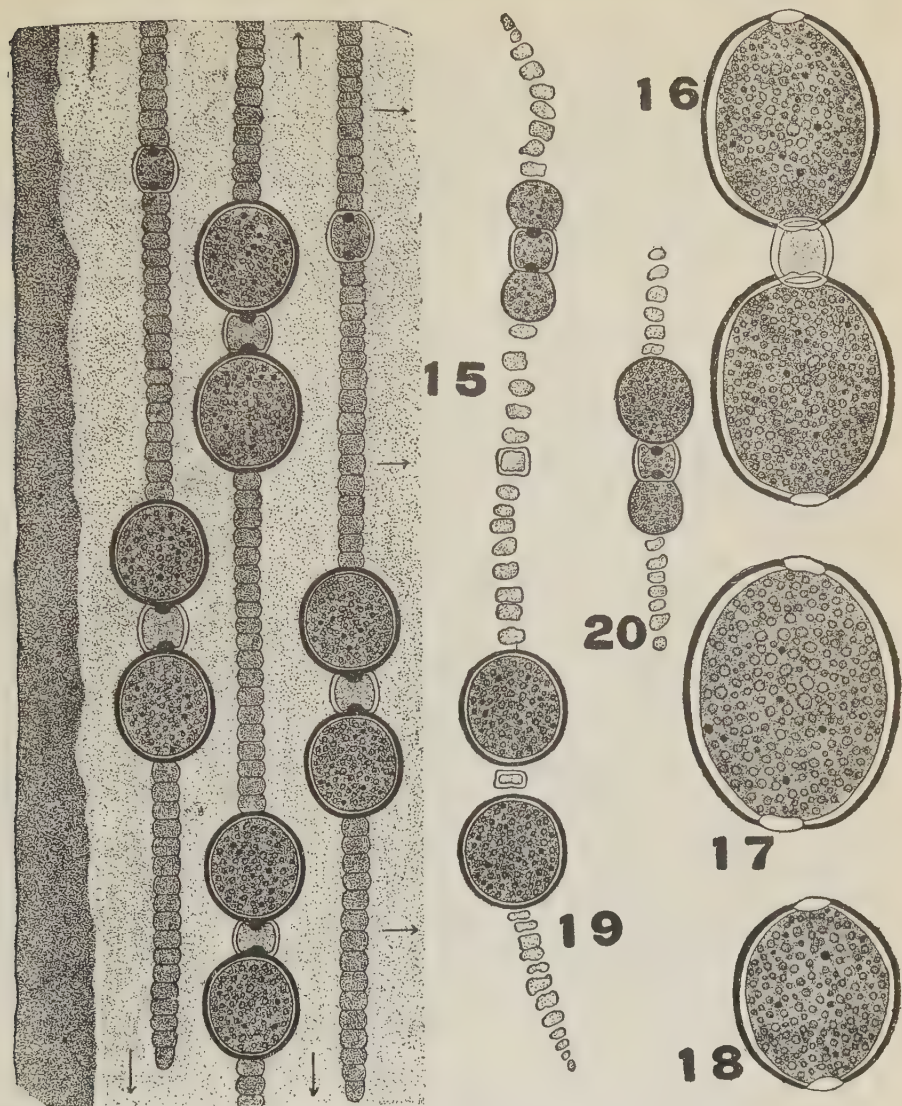


FIGS. 1-14. *Wollea Bharadwajae* sp. nov. Figs. 1-9 show the habit of the plant and the rupture of the mucilaginous sheaths at their free ends in Fig. 9. Figs. 10-12 show spore development. Fig. 13, a free trichome with mucilaginous sheath. Fig. 14, a free trichome without sheath. (Figs. 1-9 nat. size; Figs. 10-14  $\times 850$ .)

the vegetative cells and the heterocysts die, the mature spores as well as such heterocysts as are still attached to the immature spores survive (Figs. 19 and 20). The reactions of the envelopes and the contents of the spores to various stains and reagents are the same as described by Bharadwaja (1933) for *Cylindrospermum muscicola* var. *kashmirensis* and *Aulosira Fritschii*. The mature spores are generally sub-spherical, sometimes spherical, with rounded or occasionally with slightly flattened ends. They are very much larger than the heterocysts, being 14–15.7  $\mu$  broad and 17–20.6  $\mu$  long.

If the conditions are favourable some of the spores germinate soon after their separation from the trichomes, but they have never been found to germinate *in situ*. The process of germination begins with the contraction of the contents of the spore and the appearance of a small colourless papilla at its apical end (Fig. 22) or in some cases at both ends (Fig. 23). This papilla then pushes its way through the terminal pore. The papilla is followed gradually by the protoplasmic contents, the evacuated space being filled by colourless mucilage. The aperture at the end of the spore through which the contents pass out is often so narrow that they are distinctly compressed at this point (Figs. 23 and 24), recalling the form of an escaping zoospore of *Vaucheria*. As soon as a small portion of the protoplast protrudes it is cut off by a transverse wall (Fig. 25), and as the spore contents come out further more such walls are formed (Fig. 26). The empty spore shows clearly both exospore and endospore and prominently exhibits the aperture now much wider; this widening of the aperture is no doubt caused by the pressure the mucilage exerts within the spore during the liberation of the contents. The empty spore has a cup-shaped appearance, with the open edges often more or less turned back. The method of spore-germination in the alga under discussion thus agrees with that described by Fritsch (1904) as the first type of germination of spores in *Anabaena Azollae* Strasb., except that in the present alga the contents are cut off by transverse septa as soon as they are protruded, while in *A. Azollae* the transverse divisions occur after the contents have completely emerged. In regard to the germination of spores in *Anabaena Cycadeae*, Spratt (1911) writes that the 'contents are slowly protruded through a pore in the spore wall', and the author's Fig. 17 conforms with this statement. When, however, it is said further that 'The contents contract, and a colourless papilla appears at one side (Fig. 17, *b*), which advances to the inner investment (Fig. 17, *c*) and then pushes this membrane out in front of the escaping protoplast, probably to protect the latter as it passes through the exospore, during which passage it is distinctly compressed (Figs. 17, *d* and *e*)', the interpretation of the observations does not seem to be convincing, for with a pore in the spore-wall there should be no question of getting the inner investment (endospore) pushed out and its subsequent protection of the extruding protoplast. A variant method of germination from the one described above is met with in those spores of the Benares alga which are perennating and possess an exospore thickened





FIGS. 15-20. *Wollea Bharadwajae* sp. nov. Fig. 15, a part of the dissected cylindrical mucilaginous sheath, showing the parallel arrangement of the trichomes. Fig. 16, two mature spores with the intervening heterocyst, showing pores at their free ends. Figs. 17 and 18, single mature spores, showing pores at their free ends. Fig. 19, a trichome, showing the disintegration of cells other than the spores and those heterocysts which are associated with immature spores. Fig. 20, a part of the trichome showing the same as Fig. 19. (Fig. 15  $\times 850$ ; Figs. 16-20  $\times 150$ .)

all over without any trace of a pore. The spores remain dormant for one complete year and then germinate in the soil on the onset of favourable conditions. Abundant formation of mucilage takes place inside these spores, and this leads to the splitting of a part of the spore membrane more or less in the form of a lid which provides the necessary opening for the liberation of the contents (Fig. 27); the lid is thus pushed out in front of the exuding mucilage and transverse divisions of the protoplast then occur (Figs. 28-31). A similar variant has also been described by Fritsch in *Anabaena Azollae* Strasb.

#### ECOLOGICAL FACTORS DETERMINING THE FORM, VARIATION, AND OCCURRENCE OF THE ALGA

During its vegetative period, extending for a week and in which condition it is fixed by one end of its cylindrical sheath to the mud, the alga enjoys a reducing condition of the medium. The oxidation-reduction potential, as determined on a standard potentiometer (Pearsall, 1938), varies between  $E_5$  300-320 m.V. The pH value of the mud samples, as found by the electrical method, is 6.8. Comber's thiocyanate test, as modified by Misra (1938) shows that the ratio  $Fe.../Fe...+Fe..$  is 0/3 and, therefore, the reductivity is 4. A small amount of the mud used for the above measurements smeared on a white spot-plate for the diphenylamine test gives a negative result, indicating the absence of nitrates. A good deal of ammonification is to be observed in the mud as a result of the presence of a large number of bacteria. The process is quite vigorous, due to the increased temperature, rising sometimes to 45° C. during the summer months. In order to estimate ammonia in the mud samples under such circumstances, McLean and Robinson's (1924) method was employed and it was found that the quantity of ammonia was as much as 3.437 mg. per 100 gm. dry weight of the mud samples. It also conforms to the same conclusion as has been reached from the study of the above factors. These data represent the mean of a number of observations made on several mud samples taken at random from different localities in the pond and characterizing the growth of the alga. The details are given in Table I.

By the time the alga begins to form spores, the ecological conditions are slightly different, due to the gradual receding of water of the pond. The mud to which the alga is attached becomes exposed, hence the plant is exposed to a slight change of the oxidizing condition (Table II). The  $E_5$  values for the exposed mud samples vary between 350 m.V. and 365 m.V., while the pH is slightly decreased to 6.4. The ammonium thiocyanate test gives lower values for  $Fe.../Fe...+Fe..$  and the reductivity is reduced to one. The application of diphenylamine gives a slight blue colour. As the conditions become more and more oxidizing (Table II) the alga becomes detached and free-floating. The cylindrical sheaths then begin slowly to round off and finally assume a more or less ball-like appearance in which



TABLE I  
Results of Various Analyses of Mud Samples

Sample.	pH.	E <sub>h</sub> (m.V.).	E <sub>s</sub> (m.V.)	Thiocyanate.	Reductivity.	NH <sub>3</sub> (mg. per 100 gm. dry sample).
1	6.2	242	311	0/2	3	2.674
2	6.6	208	300	0/3	4	3.147
3	6.3	235	310	0/3	4	2.675
4	7.4	180	319	0/2	3	2.135
5	6.5	225	312	0/3	4	3.256
6	7.3	185	318	0/3	4	3.173
7	6.8	216	320	0/2	3	2.635
8	7.1	195	316	0/2	3	2.451
9	7.0	202	318	0/2	3	2.145
10	6.8	214	318	0/3	4	3.113
11	6.7	212	310	0/4	5	2.012
12	6.9	200	310	0/3	4	2.147
13	7.2	192	319	0/2	3	2.077
14	7.0	204	320	0/2	3	2.033
15	6.6	216	308	0/3	4	3.143

the filaments get much contorted and entangled, resembling those of *Nostoc* (Fig. 21). Some of the cylindrical sheaths may also get diffuent and allow the trichomes to move about freely in water. Most of the free-floating trichomes are naked (Fig. 14), but some show individual sheaths (Fig. 13); such a habit reminds one of a typical *Anabaena*. A comprehensive study of the water taken from different places in the pond shows that the condition existing at this time is of a perfectly oxidizing nature. The redox potential ( $E_s$ ) varies between 460 and 485 m.V., and the pH is 5.8. The reductivity appears to be nil, and the ratio  $Fe.../Fe...+Fe..$  is 2/2. The diphenylamine test gives the value for nitrates as 2, and the ammonia content of the water, as determined by the methods given in 'Standard Methods for the Examination of Water and Sewage (1936)', is nil; these data again represent the averages of a number of determinations. The details are given in Table III.

#### PERIODICITY OF THE ALGA

The periodicity of the alga is remarkably regular. As stated previously, the plant was first collected on May 25, 1939, and it reappeared exactly on the same date in 1940. In the summer of 1941, however, the pond dried up earlier, hence natural cultures of the alga were made. For this purpose soil blocks were removed from the pond without any disturbance to the surface layer and were kept in large earthen flower-pots and soaked in well-water. The water was kept 6 in. above the surface of the blocks by adding fresh water to compensate for evaporation. In these cultures the plants appeared on the same date as in 1939 and 1940. The alga, therefore, perennates by means of its spores for about a year, while the period of active life is only a fortnight or so.

TABLE II

*Results of Analysis of Mud Samples after Exposure above Water*

Exposure (hrs.).	No. of sample.	pH.	E <sub>h</sub> (m.V.).	E <sub>5</sub> (m.V.).	Thiocya- nate.	NO <sub>3</sub> .	NH <sub>3</sub> (mg. per 100 g. dry sample.)
24	1	6.2	245	314	0/1	—	1.3471
	2	6.0	254	312	0/1	—	0.4513
	3	6.3	240	315	0/1	—	0.3123
	4	6.4	234	315	0/1	—	0.1347
	5	6.3	234	309	1/1	—	0.1253
	6	6.3	230	305	1/2	—	0.1176
	7	6.4	238	319	1/2	—	0.1173
48	1	6.3	260	335	1/2	—	0.0341
	2	6.4	244	325	1/1	—	0.0254
	3	6.4	255	336	1/1	Slight	—
	4	6.4	255	336	1/1	I	—
	5	6.4	252	333	1/1	I	—
72	1	6.4	264	345	1/0	I	—
	2	6.2	276	345	1/0	I	—
	3	6.3	275	350	1/0	I	—
	4	6.2	276	345	1/0	—	—
	5	6.4	268	349	1/0	I	—
96	1	6.3	275	350	1/0	I	—
	2	6.3	275	350	1/0	I	—
	3	6.2	280	349	1/0	I	—
	4	6.4	268	349	1/0	I	—
	5	6.4	270	351	1/0	I	—
120	1	6.3	277	352	1/0	I	—
	2	6.3	270	345	1/0	I	—
	3	6.4	272	352	1/0	I	—
	4	6.2	286	355	2/0	2	—
	5	6.4	274	355	2/0	2	—
144	1	6.4	274	355	2/0	2	—
	2	6.3	280	355	2/0	2	—
	3	6.4	277	358	2/0	2	—
	4	6.4	277	358	2/0	2	—
	5	6.4	277	358	2/0	2	—
168	1	6.4	279	360	2/0	2	—
	2	6.4	281	362	2/0	2	—
	3	6.3	285	360	2/0	2	—
	4	6.2	291	360	2/0	2	—
	5	6.4	279	360	2/0	2	—
192	1	6.0	306	364	2/0	3	—
	2	6.1	302	365	3/0	4	—
	3	6.3	290	365	3/0	4	—
	4	6.4	289	370	3/0	4	—
	5	6.4	289	370	3/0	4	—

TABLE III  
Results of Analysis of Water Samples

Sample No.	pH.	E <sub>h</sub> (m.V.).	E <sub>5</sub> (m.V.).	Thiocyanate.	NO <sub>3</sub> .	NH <sub>3</sub> (mg. per l.).
1	6.0	406	464	1/1	1	0.010
2	5.8	414	460	1/1	1	0.012
3	5.8	420	466	2/2	3	—
4	5.8	416	462	1/1	1	0.011
5	5.6	426	460	1/1	1	—
6	5.8	428	474	2/2	2	—
7	6.2	416	485	2/2	2	—
8	6.2	416	485	2/2	3	—
9	5.6	446	480	2/2	3	—
10	5.8	434	480	2/2	3	—

#### DISCUSSION OF SYSTEMATIC POSITION

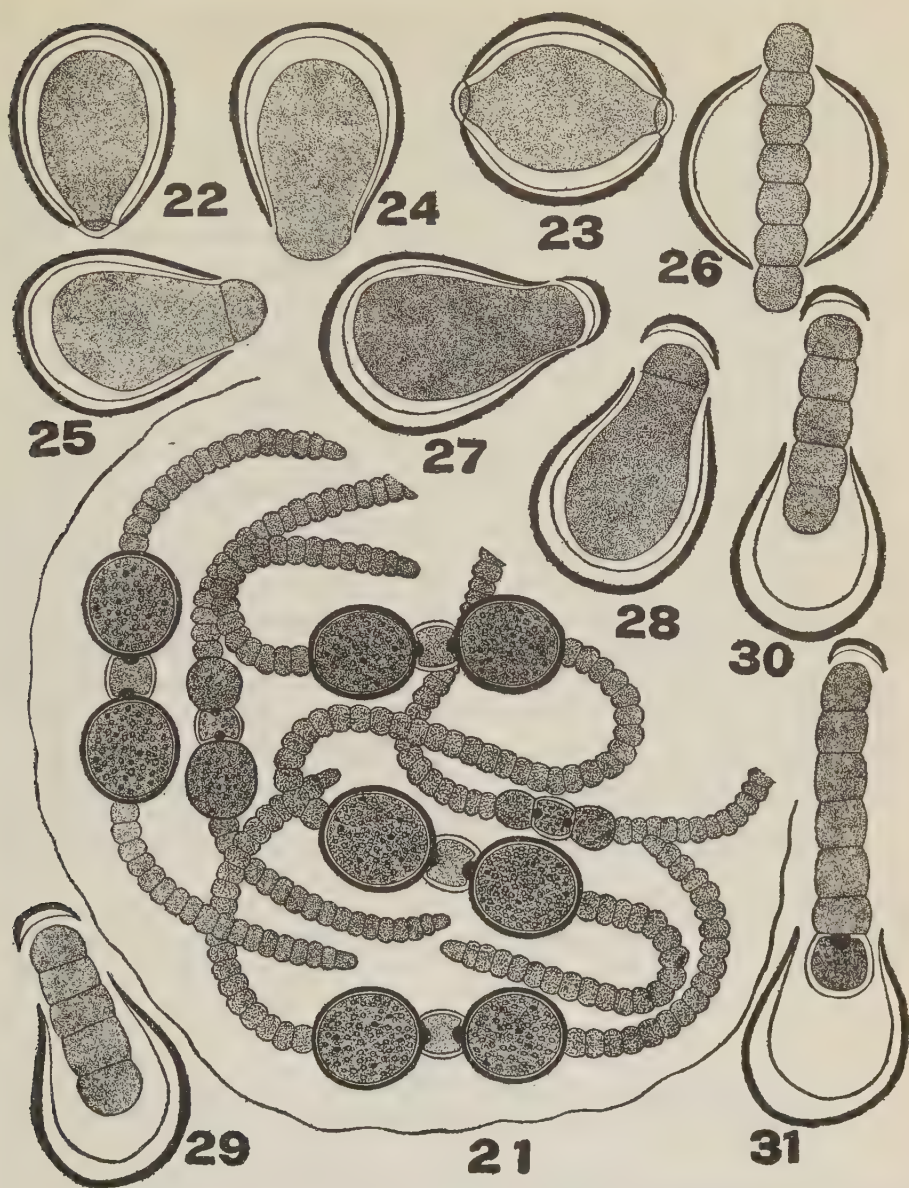
From the above account it is clear that the ecological factors are largely responsible for moulding the habit of these plants. The same plant may exhibit different appearances under different environmental conditions (cf. Bharadwaja, 1940). There are five forms, viz. (1) numerous parallel trichomes enclosed in a common cylindrical sheath fixed by one end to the mud; (2) numerous trichomes in a cylindrical sheath free-floating in water; (3) numerous trichomes, very much contorted and entangled, enclosed in a more or less ball-like mucilaginous colony; (4) individual trichomes enveloped by a sheath, and (5) free trichomes without sheath.

According to the key given by Geitler (1932, p. 803) of the genera of the Nostocaceae, the alga comes under the genus *Wolleea* Born. et Flah., on account of the sac-like colonies which the plant exhibits. Geitler (1932, p. 827) describes the genus as follows: 'Trichome überall gleich breit, gerade oder leicht gebogen,  $\pm$  parallel gelagert, in gemeinsamer, zusammenfließender Gallertmasse, zu einem weichen, schlauchförmigen Gallertlager vereinigt. Heterocysten interkalar. Dauerzellen in Reihen, neben den Heterocysten oder von ihnen entfernt', and these characters are shown by the present alga. The description of the genus as given by Geitler is based upon the study by Wolle in 1880 of a single species formerly known as *Sphaerozyga saccata*. According to the writer's investigation the important characters of the genus appear to be: (1) formation of sac-like tubular or cylindrical sheaths; (2) fixation of the plants to the soil by one end, the other end being free; and (3) numerous parallel trichomes enclosed in the sheath. But all these features are also found in the alga *Anabaena vaginicola* described by Fritsch and Rich (1930) from South Africa, and the first two features in *Anabaenothrix cylindrica*, an *Anabaena*-like alga recently described by Randhawa (1936). Again, on account of the first character it comes nearer to *Anabaenothrix epiphytica* Randhawa, another alga described by the same author (1936). A comparison in all the above main features can also be made between the present alga and *Anabaena ambigua* recently described

from this laboratory by C. B. Rao (1937). It is thus clear that the above-mentioned two species of the genus *Anabaena* and the two species of the newly established genus *Anabaenothrix*—the generic validity of which has already been questioned by Rao (1937)—agree in all essential features with the genus *Wollea* Born. et Flah. Furthermore, the Benares alga resembles *Anabaena vaginicola* Fritsch and Rich, in the rare occurrence of individual trichomes surrounded by a sheath. In the same feature it also resembles *Anabaenothrix epiphytica* Randhawa and at least 20 per cent. of the individuals of *Anabaena ambigua* Rao. This character is also present in *Anabaena Halbfassi* Bachmann, *A. inaequalis* (Kütz.) Born. et Flah., *A. Poulseniana* Boye-Pet. (Geitler, 1932, Figs. 575, 578, and 579 respectively), *Anabaena laxa* (Rab.) A. Br. (Tilden, 1910, Pl. IX, Fig. 18), and also in one other form of *Anabaena* described by Fritsch (1904). Again the Benares form resembles about 40 per cent. of the individuals of *Anabaena ambigua* Rao that are without any sheath around the trichomes. It thus appears that Fritsch, Randhawa, and Rao have overlooked the morphological importance of the cylindrical and tubular nature of the plant body and its fixation to the soil by one end, the two main features upon which the genus *Wollea* Born. et Flah. was established. Though Randhawa and Rao have figured the cylindrical and tubular colonies they have not described them. While Fritsch and Randhawa have examined their forms in preserved condition, none of the three workers has studied the complete life-history of his plant. Even Rao, who studied his form in living condition, appears to have seen *Anabaena ambigua* only in the later part of its life-cycle. Fritsch and Rao have described branching in their forms, but such a branching is hardly anything more than an accidental splitting by water currents of the cylindrical mucilage sheath at its free end (Fig. 9). It seems therefore advisable to remove the forms *Anabaena vaginicola*, *A. ambigua*, and *Anabaenothrix cylindrica* and *A. epiphytica* from the genera *Anabaena* and *Anabaenothrix* and to place them with the genus *Wollea* Born. et Flah. In the more or less ball-like mucilaginous colonies containing numerous contorted trichomes (Fig. 21), almost resembling those of the genus *Nostoc*, the Benares alga fits any of the four above forms as at present known, but it approaches *Anabaenothrix epiphytica* as figured by Randhawa (1936, Fig. 3).

As quoted by Smith (1933) the existing single species, *W. saccata* B. and F., of the genus was originally described by Wolle (1880) as *Sphaerozyga saccata* from New Jersey. Since then it has been found in Indiana (Moore, 1920), North Dakota (Moore and Carter, 1923), and Massachusetts (Collins in Phycotheca Boreali America, No. 1455). The genus is now for the first time recorded in India. A comparison of the present alga with the specific description of the type given by Wolle (1887, pp. 289–290) and also by Geitler (1932, p. 827) shows that it differs in the colonies being smaller and variable in shape in the later part of the life-cycle, the vegetative cells and the heterocysts being barrel-shaped, the trichomes being slightly tapering at the extreme

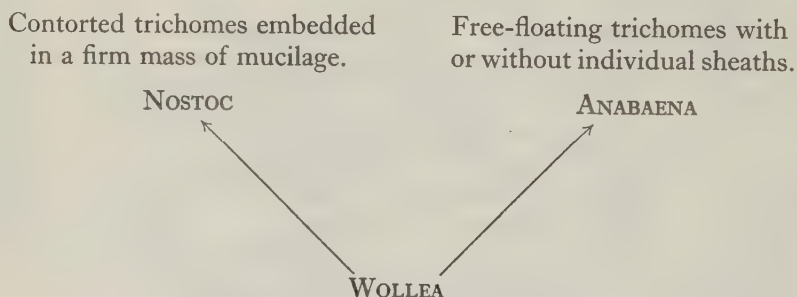




FIGS. 21-31. *Wollea Bharadwajae* sp. nov. Fig. 21, a part of the dissected ball-like mucilaginous colony, showing the contorted and entangled trichomes. Figs. 22-6, stages in spore germination in spores having pores. Figs. 27-31, the same as Figs. 22-6, in spores without any pore. (Fig. 21  $\times 890$ ; Figs. 22-31  $\times 1580$ .)

ends, and the spores being sub-spherical and formed singly on either side of a heterocyst. From *Anabaena vaginicola* Fritsch it differs in the barrel-shaped cells and heterocysts, single sub-spherical spores, and the firm nature of the colony. It also differs from *Anabaenothrix cylindrica* Randhawa in the smaller cells and heterocysts, the latter being also barrel-shaped, and in the single, sub-spherical spores; and from *Anabaenothrix epiphytica* Randhawa in the barrel-shaped cells and heterocysts and the sub-spherical single spores contiguous to the heterocysts. It contrasts with *Anabaena ambigua* Rao in the tapering ends of the trichomes, conical end-cells, barrel-shaped heterocysts, and sub-spherical spores. The present alga may therefore be considered a new species of the genus *Wollea*, and named *Wollea Bharadwajae*<sup>1</sup> sp. nov.

In the light of the present investigation it appears, therefore, that the genus *Wollea* Born. et Flah. represents a synthetic genus from which two evolutionary lines, one leading to a Nostocoid habit and another to an Anabaenoid habit, have been evolved. On the basis of the life-history of *Wollea Bharadwajae* sp. nov. it is evident that the habit of secreting and developing a common mucilage around a number of trichomes to form a gelatinous colony is more primitive than one in which the trichomes are free-floating, and that different types of habit are merely a response to different ecological and physiological conditions; possibly there is the same response in other similar algae. The genus *Wollea* should therefore be considered to be a parental stock from which *Nostoc* and *Anabaena* have been evolved (see below).



In the writer's opinion the genus *Wollea* Born. et Flah. should be defined as follows to include six species, mentioned below:

#### DIAGNOSIS OF THE GENUS WOLLEA

Plant-body in the form of cylindrical or sac-like mucilaginous structures containing numerous more or less parallel trichomes, at first fixed to the mud banks by one end, later free-floating or held up by other aquatic angiosperms. Heterocysts intercalary. Spores single or in rows, near the heterocysts or away from them.

<sup>1</sup> Named after my esteemed teacher, Professor Yajnavalkya Bharadwaja.

CLASSIFICATION OF THE GENUS *WOLLEEA*

## I. Fixed to the mud.

## A. Spores in chains.

1. Contiguous to heterocysts. *W. vaginicola*2. Distant from heterocysts. *W. saccata*

## B. Spores not in chains.

1. Spores cylindrical *W. cylindrica*2. Spores not cylindrical *W. Bharadwajae*

## II. Epiphytic.

A. Spores cylindrical. *W. epiphytica*B. Spores not cylindrical. *W. ambigua*

## SUMMARY AND CONCLUSION

The life-history and autecology of *Wolleea Bharadwajae* sp. nov. have been worked out.

The alga passes through five different types of habit during the course of its ontogeny, due to differences in the environmental and ecological conditions, i.e. the oxidizing and reducing nature of the substratum, as studied by the determination of oxidation-reduction potential, pH, base-deficiency, nitrates, and ammonia.

The position of the genus *Wolleea* Born. et Flah. in the family Nostocaceae is discussed in the light of the present investigation. The forms *Anabaena vaginicola* Fritsch, *Anabaena ambigua* Rao, *Anabaenothrix cylindrica* Randhawa, and *Anabaenothrix epiphytica* Randhawa are included in the genus *Wolleea* and a provisional classification of the genus is suggested.

In conclusion, I have much pleasure in expressing my great indebtedness to Professor Y. Bharadwaja for his kind guidance and criticism throughout the course of this investigation.

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# Studies in the Vegetative Growth and Anatomy of the Tea Plant (*Camellia thea* Link.) with Special Reference to the Phloem

## I. The Flush Shoot

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## INTRODUCTION

THESE studies were undertaken with the primary object of providing an anatomical background for investigations of the 'phloem necrosis' disease of tea in Ceylon, for which the writer was appointed as a special research officer in February 1940. The disease, which is of presumed virus origin, has been described briefly by Gadd (1939) in the Tea Research Institute's quarterly journal. No technical communication has so far been published, nor is one likely to appear for some time. Meanwhile, the anatomical work with healthy tea appeared to be of sufficient general interest to merit publication in instalments as occasion permits.

There is a considerable literature on the anatomy of the tea plant and of the commercial product. Melchior (1925), in 'Die natürlichen Pflanzenfamilien', summarizes the results of earlier investigators and gives a good account of the anatomy of *Camellia* spp. and of the family Theaceae in general. The microscopy of tea in industry is adequately dealt with in recent books by Winton and Winton (1939) and Ukers (1935). Finally, there are contributions from research workers in the principal tea-growing countries.

These include well-illustrated semi-popular articles by Tunstall and Bose (1919) and Wight (1932) from Assam, and by de Jong (1932-3) from Java. De Haan's (1939, 1941a) more detailed papers, also from Java, give the fullest account of the anatomy of the tea plant so far available.

A thorough knowledge of normal anatomy is essential for an understanding of the anatomical reactions of diseased plants. This has been emphasized recently by Esau (1938a), who has made valuable contributions to our knowledge of phloem anatomy in her studies on sugar-beet (1934) and tobacco (1938) which were undertaken to provide a starting-point for further researches on the virus diseases of curly top and mosaic. Similar anatomical problems will be presented by 'phloem necrosis' in tea. The existing literature deals with the anatomy of tea as a subject of general interest only, and provides little information on those aspects which will be dealt with most fully in the studies of which this paper is the first, namely, the developmental history of the vascular system in the growing shoot and the structure and ontogeny of the phloem tissue.

#### MATERIALS AND METHODS

The plants used were unplucked bushes about 10 years old (3 years from pruning), growing as a hedge surrounding an experimental plot on the Tea Research Institute's estate situated in the moist up-country zone at an elevation of 4,500 ft. The general type of bush would be described as a light-leaved, high *jât*, of Assam origin. *Jât* is a term only roughly corresponding to variety: the named *jâts* refer to the estate or district from which a given seed supply originates. As tea is cross-pollinated, and few if any clonal seed-bearers are available, most *jâts* consist of a large assortment of hybrids. 'High *jât*' means large-leaved, a character which serves more or less to distinguish the Assam type of bush from the 'low *jât*', very small-leaved China type.

The name *Camellia thea* Link. is adopted here in conformity with usage in Ceylon and India as defined by Wight and Barua (1939). The Java research workers employ *Thea sinensis* L., while according to Melchior (1925) and Sealy (1937) the correct name of the tea plant should be *Camellia sinensis* (L.) O. Kuntze.

Material for microtoming was fixed in formalin-acetic-alcohol and embedded in paraffin after the usual alcohol-xylol series. There was no opportunity for employing the butyl alcohol method which might have been advantageous in view of the hardness of the older woody material. The combination of safranin in 50 per cent. alcohol followed by light green in clove oil was used throughout for the permanent mounts.

Data on the periodicity of growth and flushing (see below) were obtained by twice-weekly records of tagged shoots over the period of six months from July 1941 to January 1942.

### PERIODIC GROWTH OF THE FLUSH SHOOT

The vegetative anatomy of the tea bush can be understood only in relation to the periodicity of growth that is a marked characteristic of the plant. Each vegetative shoot, and to a certain extent each bush, if left unplucked, normally goes through a rhythmic alternation of periods of active growth, involving the successive unfolding of cataphylls and foliage leaves, and of dormancy during which growth in length is completed and a marked lignification of the tissue occurs but in which no new leaves are unfolded. The active growth is described as the *flush* period (*pecco*, Dutch), the succeeding interval of dormancy as the *banji* period (*boeroeng*, Dutch). Under Ceylon conditions the regular succession of flush and banji periods proceeds to a large extent independently of the seasonal change in climate: about four complete cycles are passed through each year. Certain exceptions to this general statement are noted below.

A shoot undergoing normal vegetative growth produces a fairly constant number of appendages in each flush period. The average for the bushes under examination consisted of two bud scales, one 'fish' leaf (a small-sized foliage leaf with an entire or slightly serrate margin), and four 'flush' leaves (of the normal size and with a distinctly serrate margin)—seven appendages in all. Occasional difficulty is experienced in deciding between a bud scale and a fish leaf and between a fish leaf and the lowest flush leaf. The bud scales are usually typical deciduous cataphylls, while the fish leaves appear transitional between the bud scales and the flush leaves and are only occasionally deciduous. De Haan (1939), in Java, distinguishes between the outer bud scale, the second bud scale (or *keppel tjeuli*), and the fish leaf (or *keppel litjin*). The N. Indian term for bud scales and fish leaves collectively is *janams*. As might be expected, the degree of elongation of the internodes is least in the region of the bud scales and greatest in the flush-leaf region. In an average shoot of about 100 mm. in length the scale region will occupy 5–6 mm., the single internode below the fish leaf 10 mm., and the four flush internodes the remaining 84–5 mm. A lateral shoot usually has four or five closely crowded basal scale nodes.

The normal behaviour described above is frequently modified. Strong 'leader' shoots may show aperiodic growth, producing up to twenty or more flush leaves with normally elongated internodes without any intervening banji periods. Again, a shoot of otherwise normal periodic growth may produce a succession of flush leaves interrupted by a single deciduous cataphyll only, with a correspondingly short internode below it. At the other extreme, the terminal bud may remain banji for a prolonged period, throwing off a series of bud scales, perhaps a dozen or more, before producing a normal flush. De Haan (1941) has shown that a deficiency of nitrogen prolongs the banji period, and in fact this condition is most commonly seen in bushes of a generally unthrifty appearance. In the present account, the



anatomical relations of the normal flush shoot as defined above will be described. No attention is paid for the time being to the older stems, or to shoots in the flowering state.

The stages of development on which the anatomical work is based can now be defined. They are illustrated in Pl. IX, Fig. 1.

- A: The stage at which the flush shoot is said to 'go banji'. The uppermost flush leaf has exposed the young banji bud, which at this stage is usually about 5 mm. long and is easily distinguishable from the more elongated, rolled-up initials of the flush leaves which have preceded it.
- B: The shoot has reached its maximum length and the uppermost flush leaf its full size. This leaf and the upper part of the shoot are still flexible, i.e. are not yet fully lignified.
- C: Lignification is complete. The uppermost flush leaf is hard and brittle and has changed from light to dark green in colour. (Little difficulty was experienced in recording this stage from the 'feel' of the leaf between the fingers.) The banji bud is swollen, but normally shows no sign of breaking.
- D: The outermost bud scale has become free at the tip and along the margins, so that the bud appears to be breaking. The flush period may develop uninterruptedly from this stage, or there may be a further interval of dormancy until stage E is passed.
- E: The outer bud scale is unfolded and the second scale is free at the tip and margins. The tip of the young fish leaf may be exposed. (E<sup>+</sup>, &c., are required for successive bud scales above two in number.)
- F: The outer bud scale is commonly defoliated, the inner scale is unfolded and the rapidly developing fish leaf is emerging.
- F<sup>+</sup>: The fish leaf is fully unfolded so that the first flush leaf is exposed.
- G, G<sup>+</sup>, &c.: Mark the successive unfolding of the flush leaves until stage A is reached again as the last leaf unfolds to expose the new banji bud.

In the six months during which 70 shoots (originally 10 each of the stages A to G) were recorded, 52 shoots completed at least a single flush cycle lasting from 10 to 23 weeks. The average duration of the cycle from these data was 15 weeks, a value agreeing closely with that estimated from direct observation of the bushes as a whole. Accordingly, these shoots are regarded as a fair sample of the normal flushing behaviour of the bushes during the period of examination, and it is from them that the average flush of two scales, one fish leaf, and four flush leaves was originally determined. Of the remaining shoots, six were discarded on account of injury, and twelve passed through a part of the cycle only. These were mostly in the D and E condition for prolonged periods and produced up to eight bud scales in place of the usual two.

Table I gives a summary of the data for the duration of the stages in the flushing cycle. The descriptions refer to macroscopic appearances only. A



period of dormancy analagous to the wintering of evergreens in temperate latitudes is suggested by the condition of the shoot between stages C and E, although, as the table indicates, these stages may sometimes be merely transitory.

TABLE I

*Duration of Successive Stages of the Flushing Cycle*

Stage interval.	Average duration (weeks).	Description.
A-B	2 (1 to 3½)	'Maturation' of the previous flush.
B-C	1½ (½ to 2½)	
C-D	2½ (½ to 8½)	
D-E	3 (½ to 7)	Development and unfolding of the bud scales.
[C-E	5½ (1 to 11)]	
[E-E+, &c.	2 (½ to 6)]	
E-F	2 (½ to 6)	Unfolding of fish leaf.
F-F+	1 (½ to 3½)	
F+-G	1 (½ to 3)	Unfolding of each flush leaf successively.
G-G+		
G+, &c.-A		

THE NUMBER OF INITIALS IN THE APICAL BUD

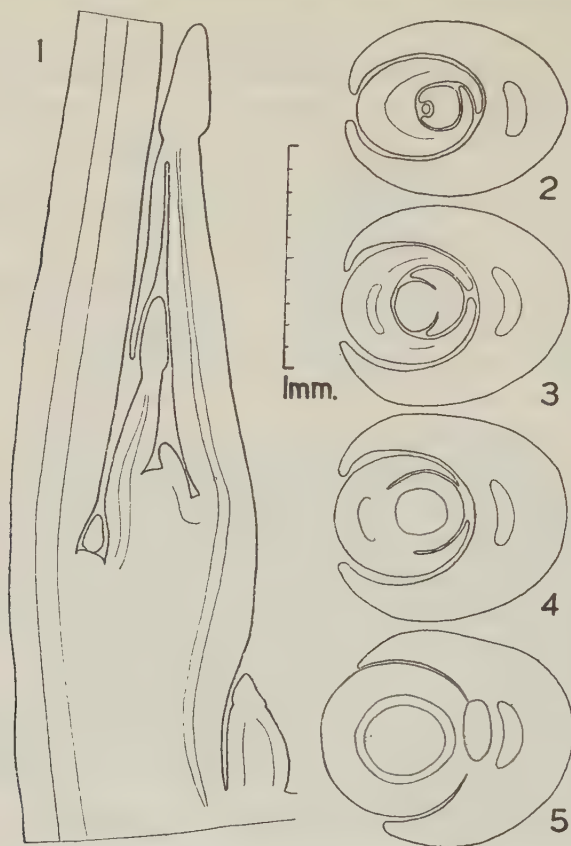
The young banji bud at its first exposure consists of three leaf-initials, i.e. the outer and inner bud scales and a simple, undifferentiated initial which will normally develop to the flush leaf. Roughly speaking, a new initial is formed every 2 to 2½ weeks, so that buds in the B, C, D, and E conditions consist on the average of 4, 5, 6, and 7 initials respectively. In other words, the full complement of leaf-initials for the average flush shoot bearing seven appendages is present by the end of the dormant phase. No exact pre-determination of the extent of the flush from the condition of the bud before emergence is implied by this statement and in fact the occasional occurrence of aperiodic growth and other variations of the normal flushing behaviour shows that no such relationship exists.

As the flush develops, further initials are laid down, and it seems likely that the bud itself then passes through a 'resting stage' during the unfolding of the last two flush leaves. In any case, no shoot has been seen in which the banji bud at its first exposure by the unrolling of the last flush leaf had more than three initials. The initials in the bud are practically distichous, but the phyllotaxy of the mature shoot is frequently much modified and difficult to determine with certainty. Each initial in the bud has a well-developed phalloid tip which appears to exert a protective function during leaf emergence, and probably also secretes slime. This tip is deciduous as maturity is reached. The structure of the apical bud immediately before exposure is illustrated in Text-figs. 1-5.

GENERAL ANATOMY OF THE FLUSH SHOOT

The general vascular structure of the stem after elongation has ceased (e.g. at the base of a young banji shoot, or at the top of the previous flush

growth at the E stage just before the new flush develops) is illustrated in Text-figs. 6–10 and in Pl. IX, Fig. 2. It consists of a closed vascular cylinder surrounded by a continuous, lignified pericycle. The cylinder is interrupted



TEXT-FIGS. 1–5. Sections of the apical bud,  $\times 15$ . The procambial areas are indicated. Fig. 1. Median longitudinal section of apex of flush shoot at stage  $G^+$  just before the unrolling of the last flush leaf (shown on the left, cut short). Figs. 2–5. Transverse sections of the newly exposed banji bud (stage A), cut at the apex and the points of departure of the third, second, and first (lowest) initials respectively.

at each node by the gap formed at the departure of the single leaf trace. The maximum width of the leaf gap is about a quarter of the circumference of the cylinder. The leaf trace contributes to the cylinder a zone of more massive development which persists through the whole length of the internode.

A brief description follows of the tissues in the mature flush stem (stage VII, below). For more complete details de Haan's (1939) paper should be consulted. It should be noted that the vascular system is described as if the tissues were in the primary condition. This seemed to be the simplest course

to adopt, although it was realized that the greater part of both xylem and phloem could be classifiable as secondary in origin. The point is considered further in the discussion.

*Epidermis.* The stem is still green and no secondary cork has been formed. The epidermis is strongly cuticularized, the cuticle being at least  $2\ \mu$  thick.

*Cortex.* The three or more outermost cell layers are collenchymatous; the innermost layer, adjacent to the pericycle, is distinguishable as a 'starch sheath'. The amount of starch present in the cells, however, is considerably greater during active elongation of the flush.

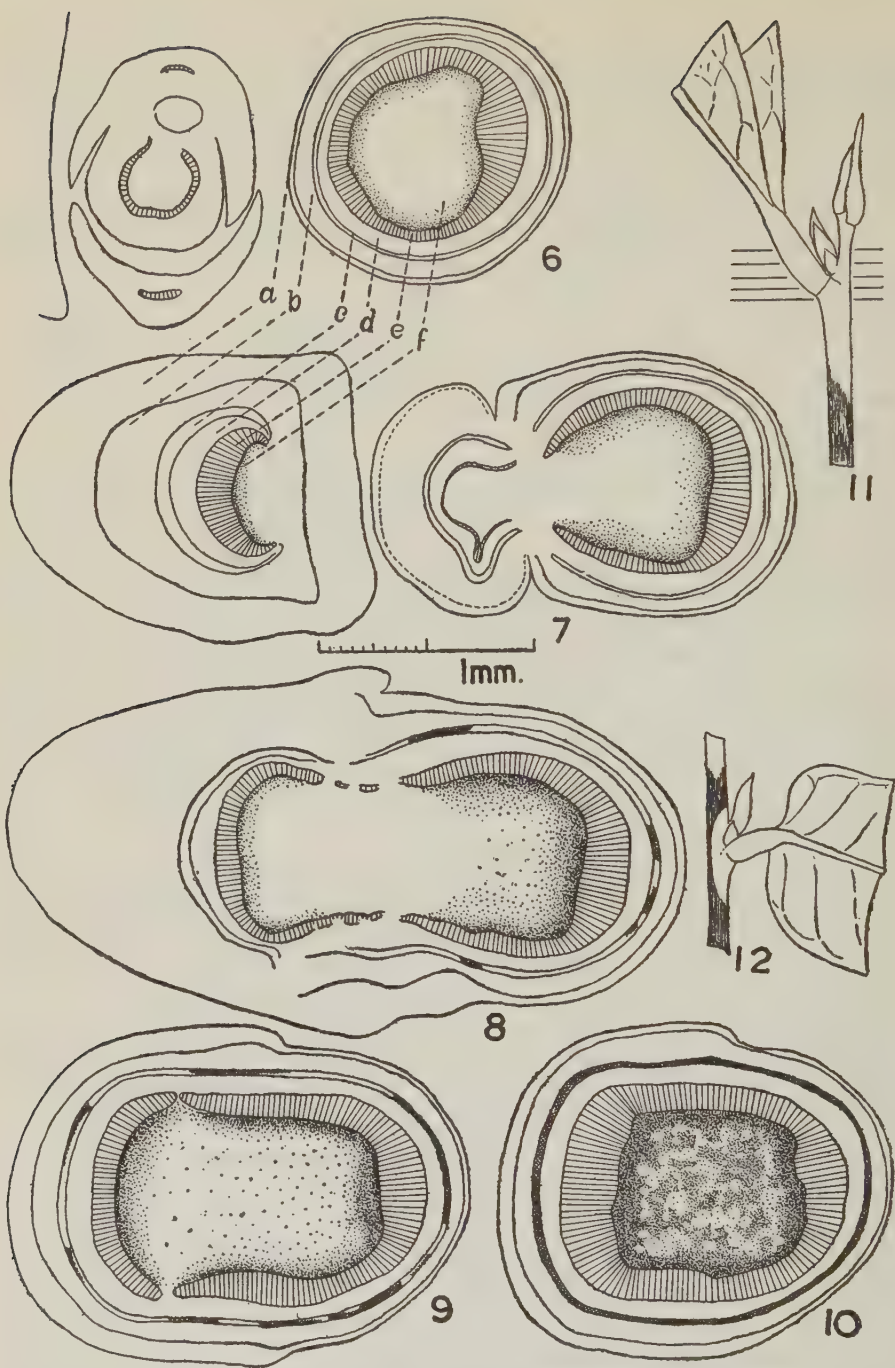
*Pericycle.* A continuous ring, 2-3 cell-layers wide, of heavily lignified fibres with simple pits and no intercellular spaces. The cells are rectangular or wedge-shaped in transverse section, averaging about  $16\ \mu$  in diameter and up to 3 mm. in length.

*Phloem.* Within the pericycle are one or two layers of undifferentiated parenchyma. The protophloem is more or less completely obliterated. A well developed metaphloem occurs. As seen in transverse section the cells are arranged in radial series, 1-6 cells wide, separated by the larger, more or less rounded cells of the medullary rays. The radial series of sieve tubes and their companion cells tend to be subdivided periclinally into 'layers' of varying width. However, as will be seen from Pl. IX, Fig. 2, and from Pl. X, the arrangement is often much modified and gives only a general impression of an orderly sequence of layers. The sieve tubes are recognized by their association with the companion cells, with deeply staining granular contents. Phloem parenchyma cells also occur. These commonly contain calcium oxalate crystals and are then arranged in long vertical rows. No lignified elements occur in the phloem.

*Cambium.* This consists of six or more cell layers which in transverse sections often show a gradual transition abaxially to a new 'tier' of sieve-tube initials. The cells are about  $15\ \mu$  broad by  $8\ \mu$  radially and of variable length owing to the occurrence of both ray and fusiform initials. The thickened radial walls and extremely thin tangential walls are characteristic.

*Xylem.* The division into narrow radial series separated by numerous, mostly one cell wide, rays is pronounced. In stems from 2.5 to 3.1 mm. in diameter, 165 to 200 xylem rays were counted. There is a gradual transition between protoxylem and metaxylem and between regions of predominantly primary and secondary type respectively. The earliest protoxylem groups on the inner face of the xylem are frequently obliterated and are in any case much stretched. Within these occur annular or spiral tracheides from 10 to  $20\ \mu$  in diameter which are gradually replaced by pitted and scalariform elements. The outer part of the xylem consists of true vessels up to  $35\ \mu$  in diameter, groups of fibrous tracheides with bordered pits, and the ray and xylem parenchyma cells which are also lignified and have simple pits. Half-bordered pits occur between the parenchyma cells and the fibrous tracheides.

*Medullary rays.* The numerous rays are chiefly one cell wide, but may be



TEXT-FIGS. 6-12. Figs. 6-10. Successive transverse sections through the mature flush node (stage VII), taken approximately at the levels indicated in Fig. 11. The tissues indicated



two cells wide in parts. Anastomoses are frequent in tangential longitudinal section, particularly in the phloem. The cells contain calcium oxalate crystals, starch, and abundant tannin. They are variable in shape, mostly more or less elongated vertically, and in transverse section appear narrowly oblong in the xylem, wider and more rounded in the phloem.

*Medulla.* The small cells of the outer part of the medulla are derived imperceptibly from the parenchyma of the innermost xylem. These cells are commonly lignified, and gorged with starch, as shown in Text-fig. 10. Towards the centre of the medulla the cells become dimorphic, groups of small starch-containing cells about 50  $\mu$  in diameter in transverse section being surrounded by large, thin-walled, and apparently empty cells of 2-4 times their size. The walls of both types of cell have numerous pits.

Large sclereids occur both in the medulla and cortex. These cells, which are characteristic in the family Theaceae, are well figured by Melchior (1925) and most of the other authors quoted. Their presence, together with the marked lignification of the pericycle, xylem, and outer medulla, makes the mature flush stem very difficult to section.

STAGES IN VASCULAR DEVELOPMENT

Seven stages in the ontogeny of the primary vascular system can be characterized, including the final stage (VII) which has already been described. Their distribution in relation to the growth of the flush is shown in Table II.

TABLE II

*Stages of Anatomical Development in Relation to the Growth of the Flush Shoot*

Position.	Growth stages.							
	A	B	C	D-E	E-F	F-F <sup>+</sup>	G	G <sup>+</sup>
10th initial							(apex)	(apex)
9th "						(apex)	—	I
8th "					(apex)	I	II	II
7th "				(apex)	—	II	II-III	III
6th "			(apex)	—	II	III	IV	IV
5th "		(apex)	—	—	III	IV	V	V
4th "	(apex)	—	I	—	IV	V	V	V
3rd "	—	—	II	III	V	—	—	VII
2nd "	I	II	III	III	V	—	—	—
1st "	II	III	III-IV	IV	—	VI	—	—
'Internode'			IV	V	V	VII	VII	VII
Uppermost flush	III-IV	IV	V-VI	VII	—	—	—	—
Second do.	V-VI	VII	VII	—	—	—	—	—

in all sections are: (a) epidermis and outer collenchymatous layers of cortex; (b) inner cortex—no starch-sheath shown; (c) pericycle—lignified where shaded; (d) phloem; (e) xylem; (f) medulla, the shading illustrates the distribution of starch. Sections  $\times 13$ . Figs. 11-12. Drawings illustrating the 'leaf-joint' nature of the petiole. Fig. 11 is from an upright branch, Fig. 12 from a branch growing horizontally, showing the plagiotropic curvature of the petiole. The brown wood region is shaded. Approx. half natural size.

The descriptions following, unless otherwise stated, are based on sections taken at the nodes and on the side of the stem opposite to the leaf, i.e. they refer to the leaf trace from the node above. The narrow lateral part of the vascular cylinder (see Text-fig. 10) may appear at least a stage behind in anatomical development. In the table, the '3rd leaf-initial' is that which would normally become the fish leaf, the 8th is the outermost bud scale of the succeeding flush, and so on. The 'internode' is the short space between the uppermost flush leaf and the outer bud scale.

*Stage I.* The apical growing-point in tea is a very small, flattened, rarely dome-shaped mass of meristematic tissue in which only the dermatogen (or single-layered *tunica*) is distinguishable as a separate layer. The distichous leaf-initials are massive and have a broad insertion. Each has a single procambial strand which unites imperceptibly with the strand from the primordium above to form a continuous ring. A 'prodesmogen' as described by Louis (1935), quoted by Foster (1936), could not be distinguished, the differentiation of the procambial strand being apparently simultaneous over the greater part of its length. The two uppermost initials join the apex adaxially almost at the same level; abaxially they are continued downwards until the emergence of the initials below in each case (see Text-fig. 1). No real distinction between nodes and internodes or between stem apex and emergent primordia can be made under these circumstances. The general impression is that of a succession of 'unit primordia', or 'phytomers' as re-defined by Evans and Grover (1940), in each of which in turn the apparent growing-point is organized anew as a lateral residue of undifferentiated meristem, soon to take its place as the next incipient member of the series.

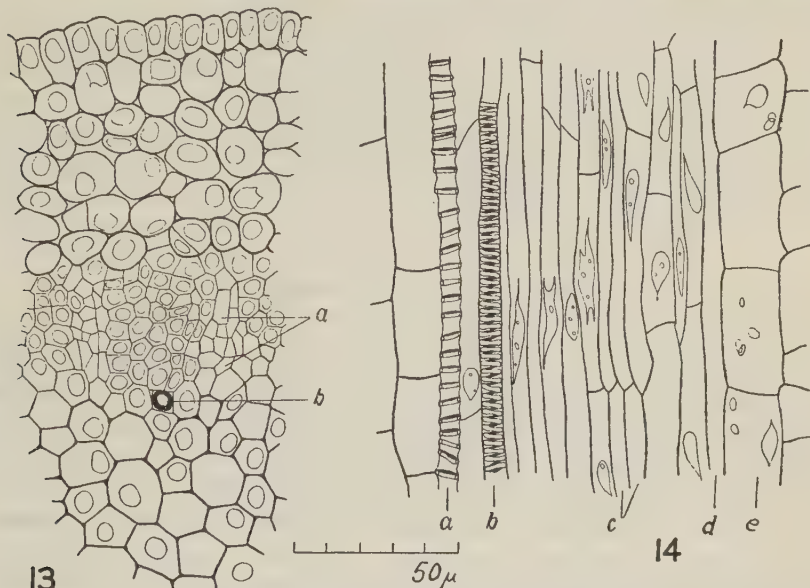
*Stage II.* Differentiation proceeds rapidly in the procambial ring and the surrounding ground tissue. Opposite the origin of the third leaf-initial from the apex (e.g. at the base of the stage A bud) the cells of the cortex and medulla are separated by intercellular spaces and the differentiation of sclereids has commenced. In the procambial ring the first protoxylem elements are distinguishable. These are spiral or annular tracheides about  $7\ \mu$  in diameter and occur singly, or rarely in twos or threes. At this stage also there is a tendency for the procambium to appear divided up into distinct 'strands' separated by narrow 'rays' one or two cells in width. A well developed strand is illustrated in Text-fig. 13. The strands are associated with the protoxylem elements where these occur, and are most distinct on the side of the stem opposite to the leaf. In favourable sections, about 25 strands in all can be counted, but in some they are barely distinguishable.

This is the only stage at which there is any suggestion of primary strands and rays. In the succeeding stages the protoxylem groups can still be distinguished, but the vascular ring itself appears continuous, and no distinction between primary and secondary medullary rays can be made.

In stage II there are no recognizable phloem elements. The procambial cells tend to be arranged in regular tiers, each about  $40\ \mu$  in depth (see Pl. X,

Fig. 4). Their end walls are more or less transverse. Certain cells on the periphery, which are narrower and more elongated than the others, are recognizable as the initials of the pericycle.

*Stage III.* The protophloem is distinguishable as small groups of immature sieve tubes with their adjacent companion cells. The origin of the vascular



TEXT-FIGS. 13-14. Camera lucida drawings,  $\times 210$ . The nuclei and some starch grains shown in outline. Fig. 13. Transverse section of young stem opposite origin of 2nd leaf-initial in stage B bud showing a single strand and adjoining rays differentiated from the procambial ring (stage II). (a) 'Ray' initials; (b) an annular protoxylem tracheide. Fig. 14. Radial longitudinal section of stem just below and on the side opposite to the insertion of the uppermost flush leaf, which has just unfolded (stage III). (a) Annular tracheide; (b) spiral tracheide; (c) sieve-tube initials; (d) pericycle; (e) starch sheath.

cambium is usually discernible. The protoxylem groups are slightly enlarged and are increased in number: from 8 to 40 groups were counted in stems 0.9 to 1.5 mm. in diameter. The differentiation and lignification of sclereids is well advanced, and the starch sheath is clearly recognizable. (See Text-fig. 14 and Pl. X, Fig. 5.)

*Stage IV.* The protophloem groups are mature (Pl. X, Fig. 6), and a well organized vascular cambium has commenced the formation of metaphloem and metaxylem initials. The ray structure is well developed, as illustrated in Pl. X, Figs. 8 and 9. The pericycle may already be recognizable in transverse sections as a 'clear layer' of cells with thin walls and no intercellular spaces. The medullary parenchyma is definitely spongy, and may be dimorphic.

*Stage V.* The first stage of obliteration in the protophloem can be seen, the cells being partly crushed and their walls thickened and contents deeply



staining. One 'layer' of metaphloem sieve tubes is mature, and the metaxylem which is rapidly expanding and becoming more heavily lignified includes elements with pitted or scalariform thickening.

*Stage VI.* This is a transitory stage, marked by the completion of growth in length and a rapid increase in width of the vascular cylinder. There is distinct obliteration of the first-formed sieve tubes, as shown in Pl. X, Fig. 7. More than one layer of metaphloem is usually recognizable and the cell-walls of the pericycle are thickened but not actually lignified. The starch sheath is less prominent, and the collenchymatous outer layers of the cortex are developing. The newly formed xylem contains vessels and fibrous tracheides. With the lignification of the pericycle, this stage passes over to stage VII, already described.

#### ASPECTS OF LEAF ANATOMY

##### *Vascular development.*

In the preceding account of the anatomy of the apical bud the leaf-initial was considered as an integral part of the 'unit primordium'. The procambial strand had developed apparently by simultaneous differentiation of the ground meristem of the primordium, at least over the greater part of its length with the exception of the apex. The subsequent development of the vascular system in the leaf-initial follows closely the differentiation of the corresponding leaf-trace region in the stem. The first-formed protophloem and protoxylem elements appear simultaneously in the trace and the lower part of the leaf. The tracheides (stage II) precede the appearance of the first recognizable sieve tubes (stage III), and the subsequent differentiation of both xylem and phloem is acropetal, i.e. from the stem and leaf base towards the apex. This course of events is apparently not typical for the majority of flowering plants studied, where, according to Esau's (1939) review, the protophloem, developing acropetally from the stem into the leaf, precedes the first protoxylem elements which originate in the leaf primordium and develop both acropetally towards the leaf apex and basipetally into the stem.

##### *Petiole structure.*

The structure of the petiole is illustrated by the diagram in Text-fig. 7, and in Pl. IX, Fig. 2, and Pl. X, Fig. 10. The fact that the entire petiole of the tea leaf is differentiated as a 'leaf-joint' has been largely overlooked in the literature. It is illustrated clearly in Text-fig. 11 and in Text-fig. 12, where the curvature of the petiole resulted from the horizontal position of the branch. This arrangement corresponds to type 2 in Funke's (1930) classification, although not all of his criteria for distinguishing 'leaf-joint' and 'normal' petiole or midrib structures are applicable to tea. The characters in best agreement with Funke's definition of a 'leaf-joint' are the extensive development of collenchyma in the external cortex and the absence of ligni-



fication in the pericycle. The latter feature, in the mature flush leaf of C or D age (see above), is in marked contrast to the heavy lignification of the pericycle and adaxial bundle-sheath in the midrib and in all but the finest veins in the lamina (Pl. X, Fig. 13).

The vascular system of the foliage leaf in tea develops in direct continuity with that of the stem throughout the life of the flush shoot. Thus, at age C onwards, the petiole bundle (except for the absence of lignification in the pericycle) shows the complete structure of the mature vascular system in the stem. This includes metaxylem and metaphloem and a well developed and apparently still active vascular cambium, as shown in Pl. X, Fig. 10. The deciduous bud scales, on the other hand, have a simple bundle with an enlarged and arrested stage III-IV structure; i.e. they consist only of proto-phloem and protoxylem and have no vascular cambium.

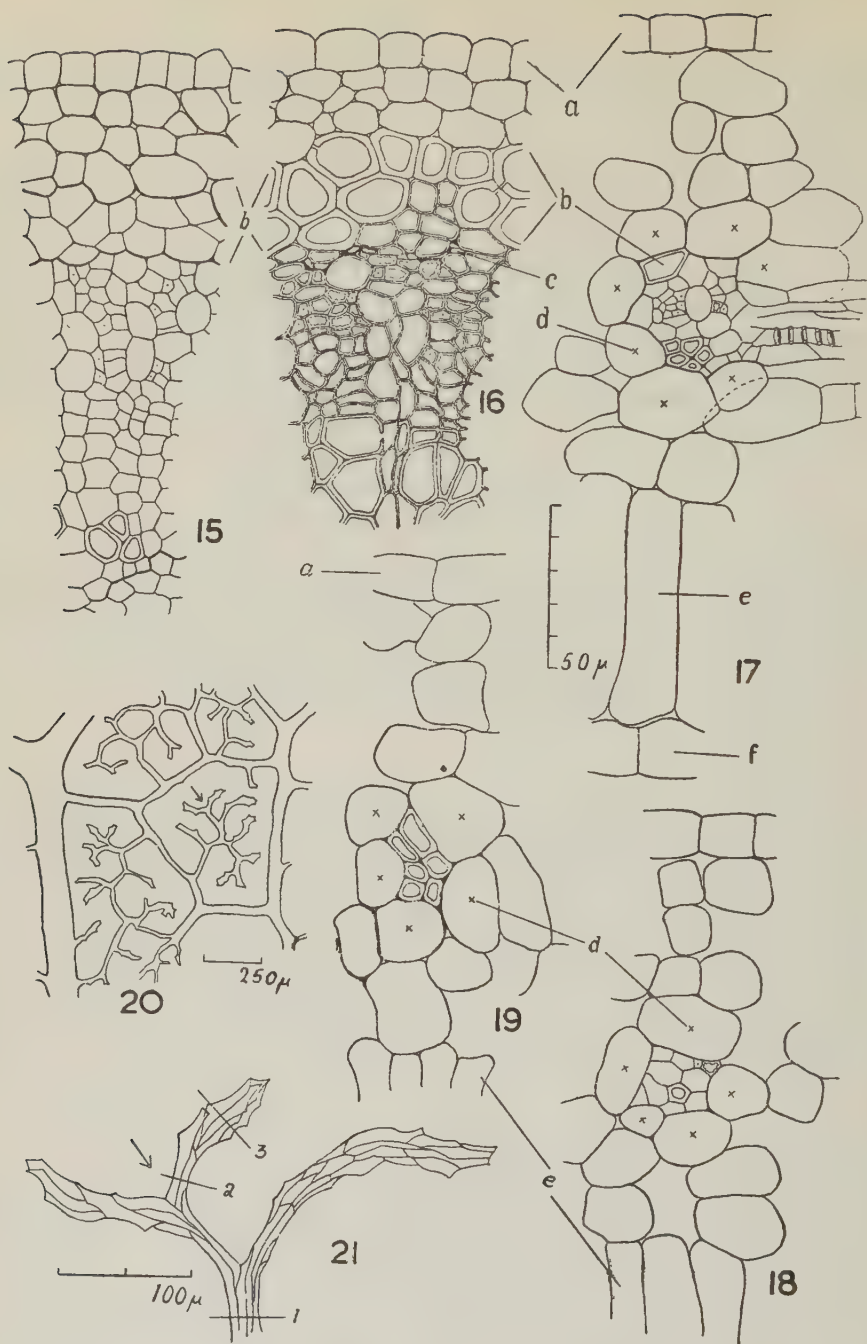
Elliott (1937) claims that the leaf xylem in angiosperms during the first year (i.e. in temperate latitudes) is continuous with the stem xylem only through the protoxylem—a condition which would appear to find a close analogy in the behaviour of the bud-scales in tea. The author quoted maintains further that in evergreens the subsequent development of the leaf trace normally occurs in complete isolation from the vascular system of the stem. Neither view finds any support in the facts as described for the flush leaf.

The subsequent history of the leaf, during secondary growth of the shoot and up to eventual leaf-fall, remains to be investigated.

#### *Veins and vein-endings.*

Sections of the midrib and lateral and marginal veins are illustrated in Pl. X, Figs. 11-13. No functional cambium can be seen in these sections, neither is the radial seriation of the phloem particularly pronounced. However, the latter is undoubtedly a 'metaphloem', with the obliterated remains of the protophloem on its abaxial surface. This point is brought out more clearly in the drawings of the marginal vein, Text-figs. 15 and 16. In the section from which Text-fig. 15 was taken there were clear indications of cambial activity. The leaf was in the rapidly expanding A condition. The marginal vein of the flush leaf is important and is illustrated rather fully because it is equivalent in size and structure to the midrib of the first few leaves of the seedling, which is frequently sectioned for diagnosis in the 'phloem necrosis' experiments.

The structure of the 'ultimate' veins and vein-endings is of obvious significance in relation to the feeding habits of possible insect vectors of virus diseases and in relation to the physiology of translocation. The disposition of the fine veins as seen in surface view in cleared material is illustrated in Text-figs. 20 and 21. It will be seen that nearly all the vein-endings are 'main endings' according to Fischer's classification, quoted by Esau (1939). The phloem associated with these veins is not distinguishable in surface view, but can be seen in section. The three sections illustrated in Text-figs.



TEXT-FIGS. 15-21. Illustrating structure of veins and vein-endings of the uppermost flush leaf: camera lucida drawings. Figs. 15-19. Transverse sections,  $\times 210$ ; the abaxial surface uppermost. The sieve-tubes are shown by their irregularly thickened walls, the companion

17 to 19 are believed to be representative of positions comparable to those marked 1, 2, and 3 respectively in Text-fig. 21. In the first position the complete vein structure is present in miniature with a small group of annular tracheides, a group of sieve tubes and companion cells with their associated parenchyma, and a single slightly lignified element representing the pericycle. Sieve plates were seen in the phloem of a portion of this bundle cut longitudinally. The second position shows a solitary tracheide (the narrowing of the xylem before the final expansion is characteristic), a single possible sieve tube, and a number of parenchymatous elements probably equivalent to the 'transition cells' described by Phillis and Mason (1933) and Esau (1939). In the final position, apparently at the actual vein-ending, the bundle consists solely of a group of somewhat enlarged tracheides. In all three sections the cells of the mesophyll immediately adjacent to the conducting elements form a loosely defined 'jacket' of border parenchyma.

#### ONTOGENY AND STRUCTURE OF THE PHLOEM

##### *Protophloem.*

The first sieve-tubes become recognizable later than the first tracheides (see above). They are differentiated acropetally in small groups usually of not more than three or four sieve-tubes and their companion cells together, and are clearly procambial in origin. The fact that their arrangement tends to be radial on the whole (see Pl. X, Figs. 5 and 6), appears to be determined merely by the general arrangement of the parenchyma cells with which they are associated. In transverse section the sieve-tubes are roughly rectangular, varying from about  $7 \times 5 \mu$  to  $10 \times 8 \mu$  in dimensions. In the leaf sections (which were not cut from embedded material) the thick cellulose walls crenulated on the inner margin were well seen, as described by Esau (1938). The lumen of the sieve-tubes is characteristically clear, or with a central dot representing the slime strand. The companion cells, which are smaller than the sieve-tubes, about 4–8  $\mu$  in average diameter, are distinguished by their densely staining granular cell contents.

Longitudinal sections of the protophloem sieve-tubes are illustrated in Text-figs. 22 to 25. In the early stages the sieve-tube and companion cell are both nucleate and of the same width, about 5  $\mu$ . The greatest length attained appears to be about 80  $\mu$ , i.e. twice the length of the procambium initials from which they are derived. The end walls are transverse or slightly oblique and form a single, poorly defined sieve-plate. Staining with aniline

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cells by a central dot. Regions marked: (a) lower epidermis; (b) pericycle; (c) degenerating protophloem; (d) parenchymatous 'jacket' cells of fine veins; (e) palisade cells; (f) upper, i.e. adaxial, epidermis. Fig. 15. Part of marginal vein, stage A leaf. Fig. 16. Same as Fig. 15, in mature, stage C leaf, showing heavily lignified pericycle and obliteration of protophloem. The same section as in Pl. X, Fig. 13. Figs. 17–19. Sections of ultimate vein, showing loss of phloem and enlargement of tracheides to vein-endings (see text). The full width of the lamina is shown in Fig. 17 to give the scale. Figs. 20–1. Surface views of ultimate venation, from whole leaf cleared,  $\times 15$ , and a part enlarged,  $\times 90$ , respectively. The positions marked 1, 2, 3 in Fig. 21 are comparable with those of the sections, Figs. 17–19, respectively.



TEXT-FIGS. 22-30. Illustrating sieve-tube structure: camera lucida drawings from longitudinal sections, all  $\times 285$ . Figs. 26-7 from fresh sections in glycerine, stained in iodine solution and aniline blue; the remainder from paraffin sections, stained in safranin and light



blue revealed the existence of callus: no 'definitive' stage was seen in these sieve-tubes, but it must be admitted that owing to their small size the details were difficult to distinguish. A single slime strand was quite easily seen.

Considerable interest attaches to the degenerative stages of the protophloem and to the origin of the pericycle. The average life of the protophloem sieve-tubes in tea appears to be from stage III, say at the third leaf-initial from the apex, to stage V, which is recognizable by the fifth or sixth initial from the apex. This represents very approximately a period of 6 weeks. At stage V, when degeneration of the protophloem is first apparent, an active cambium and well marked radial series of metaphloem and metaxylem elements are also present. In addition, there has been a general increase in size of the parenchyma cells adjacent to the sieve-tubes. Degeneration can therefore be interpreted as a simple crushing and stretching process due to cambial activity and increasing cell-size. The cells in process of obliteration develop thick walls and stain deeply and in the later stages their outlines are difficult to distinguish. They may ultimately disappear completely, or be left as local thickenings in the walls of the adjacent cells (Pl. X, Fig. 7, and Text-fig. 16). In tobacco, as described by Esau (1938), the cells surrounding the functionless sieve-tubes of the protophloem continue their enlargement and elongate rapidly to form prosenchymatous elements which on lignification of the cell-walls constitute typical 'pericyclic fibres'. The same author quotes numerous similar examples showing the origin of the so-called pericycle in the phloem. In tea, on the other hand, the pericycle is procambial in origin and its differentiation precedes that of the protophloem (stage II). It is recognizable as a distinct layer while the first sieve-tubes are still functional and is usually separated from the latter by one or more layers of parenchyma.

### *Metaphloem.*

The metaphloem as described in this paper has its origin exclusively in the vascular cambium, the differentiation of which as a distinct layer occurs

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green. Figs. 22-5 show the *protophloem* sieve-tubes. Fig. 22. The earliest recognizable elements (stage III, at the base of the young banji bud; cf. Text-fig. 14). Apparently a sieve-tube initial (which has lost its cell contents) and two companion cells are shown. There is no sign of the development of a sieve-plate on the end wall. Fig. 23. A somewhat later stage. The sieve-tube initial is still nucleate, but the formation of a sieve-plate is discernible. Fig. 24. A mature (stage IV) sieve-tube from the upper part of a stage A flush shoot. Two well developed sieve-plates and a slime strand are shown. The sieve-tubes are enucleate. Fig. 25. Degenerative stage. The cell-lumen is narrow and is easily clogged with stain. Figs. 26-30 show the *metaphloem* sieve-tubes. Fig. 26. An early mature stage. The slimy material is uncoagulated, and numerous small grains of 'sieve-tube starch' (staining red with iodine) occur. There is little callus on the sieve-plate. Starch-containing plastids are shown in the cell adjoining, and on the left is a row of parenchyma cells each with a single calcium oxalate crystal. Fig. 27. A later stage: the slime has coagulated on the sieve-plate and the 'sieve-tube starch' has disappeared. This and the remaining drawings are from *radial* sections in which the companion cell is left either above or below the sieve-tube and hence is not included. Figs. 28-30, being from paraffin sections, cannot be compared directly with Figs. 26-7. They show the gradual accumulation of callus on the sieve-plate, leading nearly to a 'definitive' stage in Fig. 30. Compare Pl. X, Figs. 14-15.

almost simultaneously with the first appearance of the protophloem elements. The radial seriation of the metaphloem depends on the orderly sequence of division in the cambium, periclinally to form the new initials, and anticlinally to cut off the companion cells (Pl. X, Fig. 10). The latter are usually displaced so as to fill the corner between two adjacent sieve-tubes. The radial arrangement and association with ray cells containing calcium oxalate crystals is well shown in Pl. X, Fig. 16. The metaphloem elements are larger in all their dimensions than those of the protophloem. Thus, the sieve-tubes in the stem, petiole, &c., are at least  $10\ \mu$  in average diameter, often swollen to  $15\ \mu$  at the ends, and have been seen up to  $160\ \mu$  in length. There is more variation in shape than in the protophloem: the general direction is straight or slightly curved, but there may be a sudden turn at the end so that the sieve-plate is almost vertical. Otherwise the end wall is transverse to fairly oblique. There is a single sieve-plate occupying at least two-thirds of the space of the end wall. The lateral walls, which are thick and *nacré* at maturity as in the protophloem, are described by de Haan (1941) as having (in the secondary phloem) small sieve-pits. These were not seen. The sieve-tubes in the vein-endings (see Text-fig. 17) are reduced in size and of a simple type comparable to that of the protophloem. The companion cells appear to be reduced proportionately.

Certain stages in the organization of the metaphloem sieve-tubes and their contents are illustrated in Text-figs. 26–30. The occurrence of ‘sieve-tube starch’ staining red with iodine, the slime strands, and the aggregations of slime on the sieve-plates were seen clearly and were found to be in general agreement with Esau’s (1939) summary. No specific ‘slime bodies’ (Esau, l.c.) occurred. The details of the progressive development of callus were not easy to follow. The maximum degree of callus-formation observed was rather less than the ‘definitive’ condition, which would place all the metaphloem sieve-tubes (i.e. in stage VII) in the ‘developmental’ and ‘mature’ stages according to Esau’s classification.

## DISCUSSION

Apart from its purely descriptive aspects, and the details of phloem ontogeny which have already been discussed, the present investigation raises several questions of wider morphological and anatomical interest. These can be considered under the general headings of (1) problems of apical growth and foliar determination, (2) vascular histogenesis.

The tea plant, with its well marked and conveniently short flush period, would be an ideal subject for studying the problem of the successive and periodic development of cataphylls and foliage leaves—a problem which Foster (1931) inferred to be pretty much at the same stage of investigation as it was a century ago. In temperate latitudes the tendency to periodic growth can hardly be considered apart from the yearly succession of climatic

change, but at least under Ceylon conditions it here presents itself as apparently an inherent one, modifiable but not entirely controlled by climatic factors.

A profitable comparison may be made with Foster's (1931a, 1932) data on the behaviour of the Black Hickory (*Carya Buckleyi* var. *arkansana*). The spur shoots of this plant were found to produce a fairly constant annual 'flush' of 2 outer bud scales, termed 'upper transitional forms', 7 cataphylls, and 4 foliage leaves: 13 appendages in all. Examination of the dormant apical bud showed that not only was the entire growth for the year pre-formed and recognizable before emergence, but that in addition the initiation of the next season's terminal bud, in the form of the two upper transitional forms, had commenced in most cases. At the first exposure of the new bud, in the middle of April, at least eight primordia were present, the ninth following within a few days. It was emphasized that the completion of scale formation in the new bud appeared to coincide with the general cessation of growth of the main shoot. In tea, the new flush (but not the two bud scales following) is pre-formed before active elongation commences and at least the scale and fish-leaf primordia are completed before it ceases. The slight divergence may perhaps follow from the less pronounced resting period and the much smaller number of bud scales. Otherwise, much the same state of affairs holds good for the tea flush as for the yearly growth of the deciduous tree. The nature and direction of this apparently causal relationship between the condition of the bud and the state of growth of the shoot cannot be considered here.

Scale formation is usually, and quite naturally, held to coincide with an arrest of growth and the excessive production of cataphylls in tea under conditions of nitrogen deficiency appears to support this view. In fact, the behaviour of the apical bud in laying down new initials must be kept apart from the subsequent behaviour of the developing shoot. Foster's (1932) data for black hickory show that the scale primordia may actually be laid down at a faster rate than the leaf primordia: the plastochron being 4 days for the former as against  $5\frac{1}{2}$  days for the latter. But in this species the period of active formation of primordia of both types appears to be confined to about fifty days in the year, the apical bud being dormant from about the middle of May until the renewal of activity in March. In tea, on the other hand, the activity of the apical bud is more nearly continuous, and preliminary calculations indicate a relatively constant plastochron for all primordia of the order of 2 to  $2\frac{1}{2}$  weeks. Further investigations are needed on this point.

Inseparable from the above considerations is the problem of foliar determination. Foster's papers, already quoted, support the view that the primordia in the apical bud are 'determinate', i.e. that their future development either into cataphylls or foliage leaves is predictable, from a very early stage. The same author (1931) also refutes the tendency to accept the occurrence



of foliar appendages of intermediate type as evidence in favour of the still current interpretation of the cataphyll as a 'reduced' foliage leaf. Here also the tea flush presents excellent opportunities for study in the more or less constant appearance of the fish leaf and in the marked contrast in the mode of origin of the vascular system of the cataphylls and foliage leaves, entirely procambial in the former and involving an active vascular cambium in the latter.

The study of tea anatomy raises the same problems of vascular histogenesis as were discussed by Esau (1938) in her study of tobacco phloem. These are, briefly, the distinction between primary and secondary tissues, the problem of leaf-trace differentiation, and the origin of the pericycle. The last two have already been discussed sufficiently in the text and only the first need be considered here. It may be noted at the outset (Esau, l.c.) that the vascular system in tobacco consists, as in tea, of a closed ring interrupted only at the departure of the single leaf trace. The occurrence of internal phloem in tobacco has no significance for the present discussion.

Esau showed that the difficulty in distinguishing between primary and secondary tissues was partly a terminological one. She distinguished them according to their origin respectively from *procambium* and *cambium*. The procambium, which was active mainly in elongating organs, had the following characteristics (Esau, 1938, p. 397): 'more or less polygonal cells in transverse sections; scarcity of oblique end walls; lack of sharp distinction between initials of transverse and longitudinal systems; absence of oblique anticlinal divisions.' The cambium, on the other hand, was active in regions that had ceased to elongate and was characterized by: 'more or less rectangular cells in transverse sections; presence of distinct ray and fusiform initials; occurrence of oblique anticlinal divisions' (l.c., p. 398). The occurrence in the cambium of comparatively thick radial walls and very thin tangential walls was also noted. The usual adoption of radial seriation of the cells as the principal criterion for cambium could not be upheld, since this occurred also (but to a less marked extent), in the procambium.

With Esau's morphological distinctions between procambium and cambium the writer is in complete agreement, and in the present paper the terms 'procambium' and (usually) 'vascular cambium' connote respectively meristems equivalent in structure and appearance to those described in tobacco by the author quoted. In tea, however, the procambium is superseded by an unmistakable vascular cambium while the stem is still actively elongating. A large part of the tissues produced by the vascular cambium up to the mature stage of the green shoot characterized by the cessation of growth in length and the lignification of the pericycle (stage VII, above) may therefore be described as primary in origin. There is no dividing line between primary and secondary tissues as such, since both result from the same meristematic process. On the other hand, there is a well marked distinction between the mature green stem, as described above, and the 'brown wood' region charac-



terized by the formation of corky periderm from a phellogen layer situated within the pericycle, as described by de Haan (1941a) and others. The cessation of growth in length and concomitant lignification of the pericycle is thus seen in its true perspective as the concluding phase of primary growth, while the initiation of phellogen activity marks the beginning of the secondary stage. The fact that the transition between primary and secondary growth frequently coincides with the transition from the procambial to the cambial type of meristem has led to the belief that a necessary relationship exists between them. The development of the vascular system in tea shows that such is not the case. The distinction between procambium and cambium should be left as a purely anatomical one: the distinction between primary and secondary growth as a developmental one involving the cessation of growth in length and the appearance of the phellogen. All the tissues described in the present paper are 'primary' on this interpretation. Among them, it has been convenient to distinguish those vascular elements of clearly procambial origin as *protophloem* and *protoxylem*, and those originating from vascular cambium as *metaphloem* and *metaxylem*, respectively, although no wider application is claimed for this distinction.

#### SUMMARY

The present communication deals with the anatomy and periodic growth of the young, unplucked shoots of the tea bush (*Camellia thea* Link.) growing in the moist up-country zone of Ceylon at an elevation of 4,500 ft. The type of bush is described as a light-leaved, high *jât*, of Assam origin. The meaning of these terms is defined.

The normal growth period or 'flush' involves the successive unfolding of two scale leaves, one transitional 'fish' leaf, and four foliage leaves. It is succeeded by the 'banji' period, during which growth in length is completed and considerable lignification occurs, but in which no new leaves are unfolded.

The 'banji' bud at its first exposure consists of three primordia. This number increases to seven by the beginning of the flush, i.e. the average complement of appendages for the flush is pre-formed and recognizable before active elongation commences. However, there is no effective pre-determination of the extent of the flush which is universally valid since variations in the normal flushing behaviour occur which cannot be predicted from the bud. In particular, the shoot may undergo 'aperiodic' growth involving the continued production of foliage leaves with no intervening banji periods. These considerations are discussed in relation to modern concepts of apical growth and 'foliar determination'.

The general vascular structure of the flush shoot after elongation has ceased is that of a closed cylinder interrupted only by the departure of the single leaf trace. The very numerous rays are mostly only one cell wide. Both xylem and phloem elements show a well marked radial seriation, and

an active cambium is present. The vascular cylinder is surrounded by a continuous lignified pericycle.

The apical bud is interpreted as a succession of 'unit primordia' in each of which a single, broad procambial strand is differentiated. The differentiation of the strand appears to be simultaneous, at least over the greater part of its length. The procambial strands early extend to form a continuous ring, in which the differentiation of the protoxylem elements precedes the appearance of the first recognizable sieve-tubes of the protophloem.

The cambium is differentiated while the stem is still elongating, and its products are here interpreted as metaxylem and metaphloem. The usual definition of *procambium* and *cambium* as the meristems giving rise to *primary* and *secondary* tissues respectively cannot be upheld in the tea plant. It is better to retain the distinction between the meristems as a purely anatomical one involving differences in shape, disposition, &c., of the dividing cells. The distinction between primary and secondary tissues then becomes solely developmental. All the tissues here described are conceived as primary in origin since they are laid down before stem elongation ceases and before the initiation of phellogen activity and the formation of a periderm.

The protophloem is almost entirely obliterated in regions that have ceased elongating. The obliteration process is not associated in any way with the development of the pericycle, which is recognizable as a distinct layer while the first sieve-tubes are still functional, and which is considered to be entirely procambial in origin.

The sieve-tubes of the metaphloem are characterized by their more or less orderly arrangement in radial series subdivided periclinally into layers. There is a single sieve-plate on the end wall which is transverse to oblique. The occurrence of 'sieve-tube starch', slime, and the progressive development of callus is noted.

The vascular development of the foliage leaf follows closely the development of the corresponding leaf-trace region of the stem, the petiole bundle of the mature leaf having a well marked cambium and radially seriated xylem and phloem elements.

The deciduous bud scales, on the other hand, have a simple vascular organization consisting only of protoxylem and protophloem elements of procambial origin.

Reasons are given for interpreting the entire petiole as a 'leaf-joint' structure. The pericycle remains unligified here, but is strongly lignified in the midrib and in all but the finest veins.

The structure of the 'ultimate' veins and vein-endings is described. No sieve-tubes occur for a short distance behind the actual vein-ending which consists only of a group of tracheides somewhat enlarged in diameter.

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## EXPLANATION OF PLATES IX AND X

Illustrating Dr. T. E. T. Bond's paper on 'Studies in the Vegetative Growth and Anatomy of the Tea Plant (*Camellia thea* Link.) with Special Reference to the Phloem. I. The Flush Shoot.'

## PLATE IX

Fig. 1. Growth stages of the tea flush. *Top row*, left to right, A, B, C, D. Second row, left to right, E, F, F<sup>+</sup>, G. In C to G the uppermost leaf of the previous flush (the 'banji leaf') is cut short. Scale scars marked *ss*, the fish leaf, *f*. ( $\times \frac{1}{3}$  approx.)

Figs. 2-3. Transverse sections of mature flush stem (stage VII) and 'banji leaf' petiole (stage C) respectively. Cambium marked *c*, phloem *ph*, pericycle (lignified in stem but not in petiole) *pc*. ( $\times 100$ .)

## PLATE X

All are photomicrographs of sections stained in safranin and light green. Figs. 4-9 illustrate the stem anatomy, Figs. 10-13 the leaf ('banji leaf', stage C-D), and Figs. 14-16 the details of the metaphloem sieve-tubes.

Fig. 4. Stage II-III. Radial longitudinal section at base of second leaf-initial in 'B' bud showing protoxylem tracheides and the successive tiers of procambium cells. ( $\times 325$ .)

Fig. 5. Stage III. Transverse section, somewhat below Fig. 4, showing the protoxylem elements below, the origin of the vascular cambium in the centre, and the first protophloem sieve-tubes and their companion cells (marked with arrows) above. (Taken with a  $\frac{1}{2}$ -in. oil immersion objective,  $\times 850$ .)

Fig. 6. Stage IV, slightly below Fig. 5. The protophloem groups are mature. Above them are the 'clear cells' of the pericycle and the starch sheath or innermost layer of the cortex (both marked). ( $\times 350$ .)

Fig. 7. Stage VI, at base of expanding flush. The cells marked are: centre, a group of protophloem elements in an advanced stage of degeneration; above, the thickened but still scarcely lignified pericycle. ( $\times 350$ .)

Figs. 8-9. Tangential longitudinal sections illustrating the structure of the medullary rays (stage IV-V). Fig. 8, passing just within the cambium, shows a group of ray initials with the young, fusiform, unthickened elements on the outer edge of the metaxylem. Fig. 9, passing through the metaphloem, shows the broad ray cells (the densely staining ones filled with tannin) with narrow conducting elements between them. (Both  $\times 350$ .)

Fig. 10. Transverse section of central part of the petiole bundle, showing the cambium and pronounced radial seriation of the metaphloem elements. (Hand section,  $\times 300$ .)

Fig. 11. The midrib bundle. In the top right-hand corner is a portion of the lignified pericycle. Traces of a cambium, probably no longer active, are shown in the centre. ( $\times 300$ .)

Fig. 12. A lateral vein bundle. The section extends to the abaxial epidermis. Note the lignified pericycle (some of the cells still nucleate) and the collenchymatous cortical layers. ( $\times 300$ .)

Fig. 13. A low-power section of the marginal vein, part of which is illustrated also in Text-fig. 16. Both the pericycle and the adaxial bundle-sheath are strongly lignified. ( $\times 100$ .)

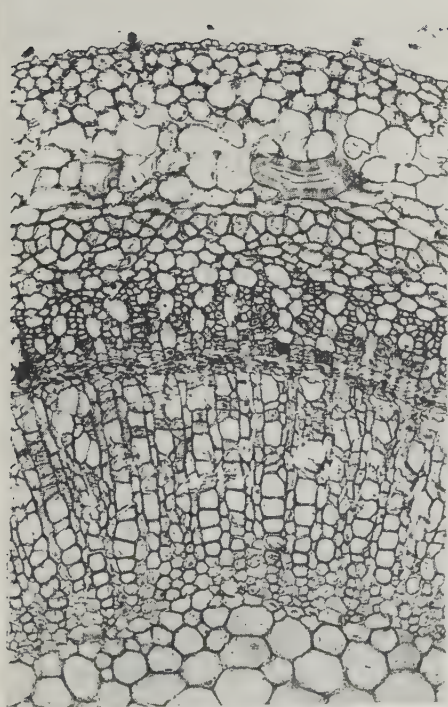
Figs. 14-15. Mature metaphloem sieve-tubes (stage VII) of stem in longitudinal section, showing slime-aggregates above sieve-plate and the formation of callus below (cf. Text-figs. 28-30). (Taken with  $\frac{1}{2}$ -in. oil immersion objective.  $\times 850$ .)

Fig. 16. The same, in transverse section. Two radial series of metaphloem elements are shown, above which is a part of the degenerating protophloem. The sieve-tubes mostly have a central dot representing the slime strand. ( $\times 735$ .)

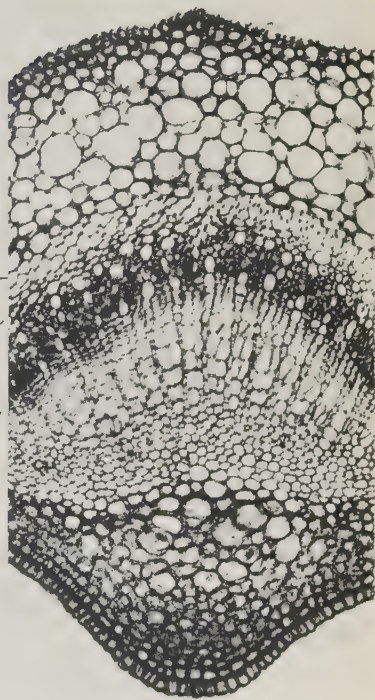




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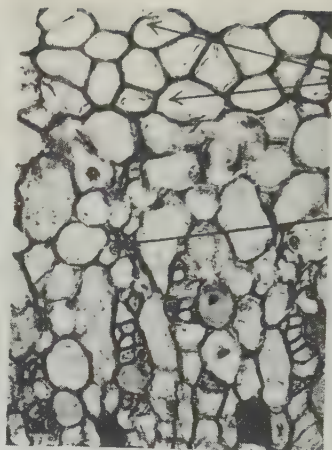
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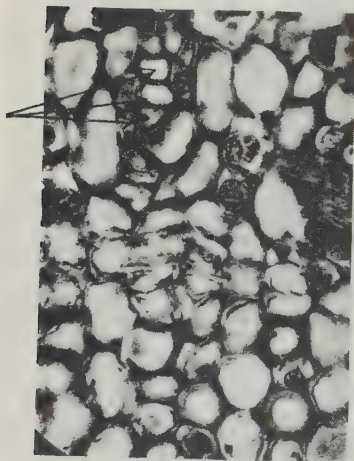
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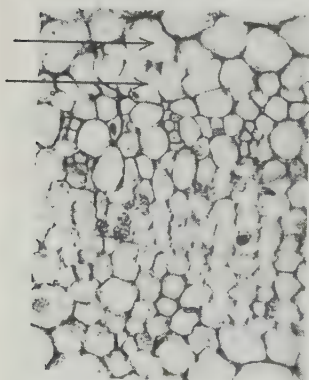
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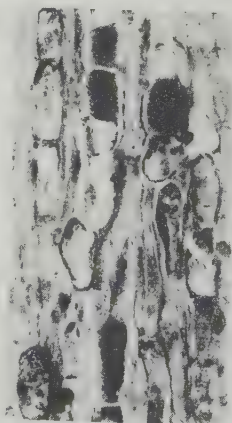
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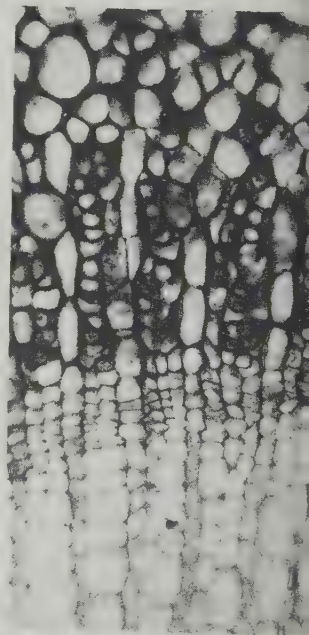
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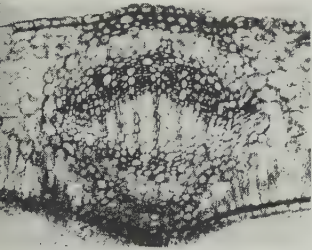


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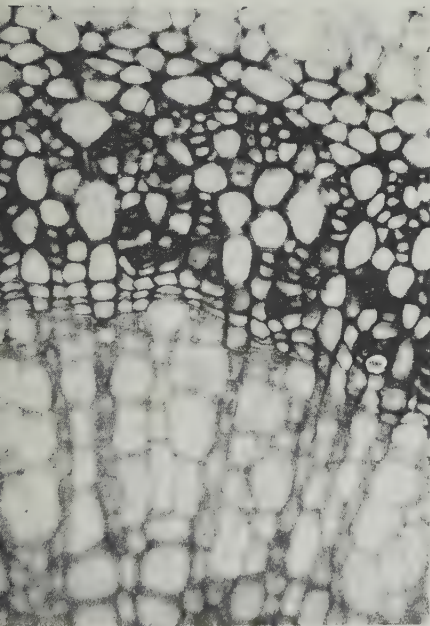




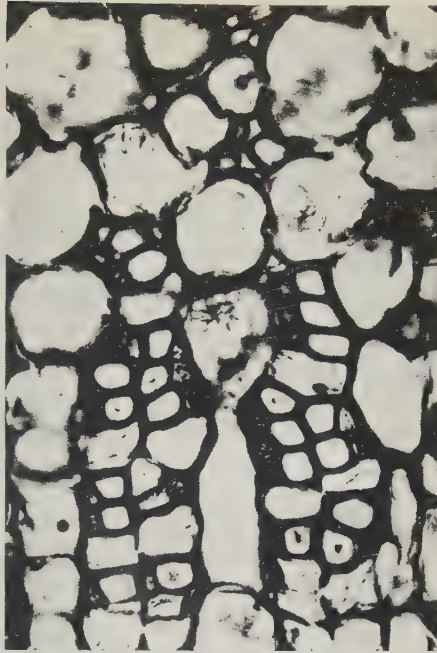
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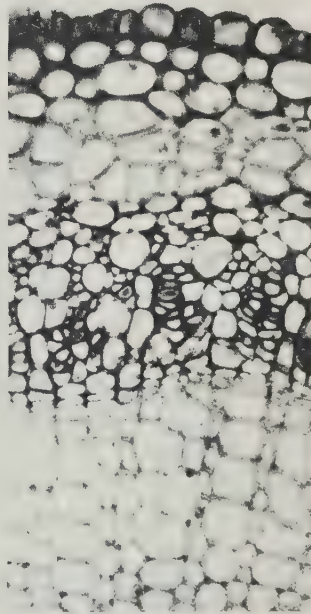
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# The Effect of Vitamin B<sub>1</sub> on the Concentration of Glucose Optimal for the Fruiting of Certain Fungi

BY

LILIAN E. HAWKER

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**D**URING an investigation of the factors influencing the production of perithecia by *Melanospora destruens* it became clear that the three most important were the presence or absence of certain accessory factors or growth substances, the concentration of glucose or other carbohydrate, and, to a lesser extent, the rate of production of toxic staling substances (Hawker, 1936). In early experiments the necessary growth substances were supplied in the form of media on which certain other fungi, capable of synthesizing these substances, had been grown (Asthana and Hawker, 1936) or of a crude extract of lentils found by Buston and Pramanik (1931) to promote the growth of *Nematospora gossypii*. Later (Hawker, 1939 and 1939a) it was demonstrated that biotin—first isolated by Kögl and Tönnis (1936) and recently identified with vitamin H by György et al. (1940)—was necessary for mycelial growth of *M. destruens*, while perithecia were only formed when vitamin B<sub>1</sub> (aneurin of European and thiamin of American workers) was added. It was shown that the increase in fruiting caused by the addition of vitamin B<sub>1</sub>, either as a pure preparation or in the form of the crude extract or 'staled' medium,<sup>1</sup> could be reduced or prevented by the addition of sufficient glucose to the medium. Moreover, the concentration of glucose optimal for perithecial production rose with increasing concentration of the vitamin. Two other fungi, *Zygorhynchus moelleri* and *Sordaria fimicola*, were shown to behave in an essentially similar manner when the source of growth substance was 'staled' medium or lentil extract (Hawker, 1936), but pure vitamin B<sub>1</sub> was not then available in large quantity.

A similar investigation of other fungi seemed likely to be of interest, and accordingly preliminary experiments were carried out in this laboratory by Keyworth (1941) with *Coprinus ephemerus* and by Mounsey-Wood (unpublished data) with *Phytophthora cactorum*. The former used a basic medium containing sodium nitrate, dipotassium hydrogen phosphate, and glucose. In two experiments the concentrations of glucose optimal for fruiting on this

<sup>1</sup> As in earlier work a 'staled' medium is one in which a fungus has been grown for a short time but not long enough for the concentration of toxic staling substances to have become inhibitory.

medium in the absence of any external supply of growth substances were 0.8 and 1.5 per cent. The addition of 0.5 per cent. (dry wt.) crude lentil extract raised these optimal concentrations to 2–2.5 and 2–4 per cent. respectively. A similar effect was obtained when the source of growth substance was 'marmite' or a pure preparation of vitamin B<sub>1</sub>. Mounsey-Wood grew *P. cactorum*, which requires an external supply of vitamin B<sub>1</sub> (Robbins, 1937), on a modification of Petri's medium in which the source of carbon was fructose and to which various quantities of lentil extract or of a pure preparation of the vitamin were added. The concentration of fructose optimal for the production of oogonia was raised as the quantity of growth substance was increased.

The writer then undertook an investigation of a wider range of fungi. The scope of the work has been limited by the need to conserve materials, but the results obtained are sufficiently uniform to warrant publication.

#### EXPERIMENTAL METHODS

The basal medium was that used in previous work with *M. destruens* (viz. medium A; glucose, concentration varied; KNO<sub>3</sub>, 3.5 gm.; MgSO<sub>4</sub>, 0.75 gm.; KH<sub>2</sub>PO<sub>4</sub>, 1.75 gm.; agar, 15 gm.; water, 1 litre) unless otherwise stated. Vitamin B<sub>1</sub> was added in the form of crude lentil extract or as a pure preparation. Some of the fungi used were unable to grow in the absence of biotin (Hawker, 1939a). This was added in the form of 0.05 per cent. lentil extract, since sufficient pure biotin was not available. The concentration of glucose in the extract was estimated by Bertrand's method, and that of vitamin B<sub>1</sub> by comparison of growth of *Phycomyces nitens* (Schopfer and Jung, 1937, and Hawker, 1939a) in the presence of the extract with that in the presence of pure vitamin B<sub>1</sub>. Allowance was made for these concentrations when adding glucose or vitamin B<sub>1</sub> to media containing lentil extract.

The majority of the experiments were carried out in petri dishes of 9 cm. diameter and were replicated five times. Later, however, in order to conserve materials experiments were in triplicate and smaller dishes were used. The fact that the results obtained were all of the same order made this reduction possible. Certain species of Basidiomycetes were grown on thin layers of agar medium in medicine bottles (plugged with cotton-wool and laid on their sides), since the risk of contamination in petri dishes was considerable owing to the relatively long duration of these experiments. Cultures were incubated at 20–25° C., with the exception of fungi requiring light for fruiting which were exposed to the light in a north window at room temperature in summer or in a heated greenhouse in winter.

The amount of fruiting was estimated by the method of Asthana and Hawker (1936) where this could be applied. In some cases excessive mycelial development, aggregation or variation in size of the fruit bodies made accurate counting impossible and arbitrary values were used. Such methods will be described in the appropriate places.



Detailed results of experiments with *M. destruens* have already been published (Hawker, 1936, Tables IV and V, and Hawker, 1939a, Tables IV and V). With low concentrations of lentil extract growth was poor and perithecia were formed only when the percentage of glucose was very low. With larger amounts of the extract growth was increased, particularly with relatively high concentrations of glucose, and the percentage of glucose optimal for fruiting was raised. When the concentration of lentil extract was kept low and pure vitamin B<sub>1</sub> was added the increase in mycelial growth was much less marked, but the concentration of glucose optimal for fruiting was again raised. Growth in this instance was probably limited by the small amount of biotin present in a low concentration of the extract. Since the latter contains both biotin and aneurin, it was not possible in the absence of a supply of pure biotin to increase the amount of this substance without also increasing that of vitamin B<sub>1</sub>. A single small-scale experiment with pure biotin (already reported in the earlier paper) indicated that mycelial growth increased with increase in concentration of this substance which, however, did not alter the amount of glucose optimal for fruiting.

*Melanospora zamiae*, *Podospora curvula*, *Philocopra* sp., and *Sordaria fimicola*<sup>1</sup> were examined in less detail than *M. destruens* and gave essentially similar results, viz. the concentration of glucose optimal for perithecial formation was raised by increasing that of lentil extract or of pure vitamin B<sub>1</sub>.

*Chaetomium cochloides* and *Pyronema confluens* were both able to fruit in the absence of any external supply of growth substance, but did so more freely with the addition of vitamin B<sub>1</sub>. With both fungi, as with those already described, the concentration of glucose optimal for fruiting rose with increasing concentration of the vitamin. Mycelial growth was very great with high concentration of glucose making accurate counts of fruit bodies impossible, but the differences observed were sufficiently large for the optimum concentration of glucose at any given concentration of vitamin B<sub>1</sub> to be determined accurately.

*Collybia velutipes* produced only a few small fruit bodies in the absence of an external supply of aneurin. These were produced on or near the inoculum, which probably contained a small amount of the vitamin, and with high concentration of glucose none was formed. If lentil extract or pure vitamin B<sub>1</sub> was added, growth and fruiting were both increased, this being most marked with higher concentrations of sugar. As in the case of the ascomycetous fungi already described, the concentration of glucose optimal for fruiting rose with increase in concentration of aneurin. Similar results were obtained with *Hydnum coralloides* and *Schizophyllum commune*, while Keyworth's (l.c.) results with *Coprinus* were also in agreement. With all these Basidiomycetes the size of the fruit bodies varied so greatly that an arbitrary

<sup>1</sup> The strain of *S. fimicola* had ceased to produce perithecia before a pure preparation of vitamin B<sub>1</sub> was available, hence results with this fungus refer to concentration of lentil extract only.

TABLE

	Concentration of vitamin B <sub>1</sub> ( $\gamma$ per 100 c.c. medium).				Concentration of lentil extract (g. (dry wt.) per 100 c.c. medium).			
	1	2	4		0	0.05	0.1	0.2
<i>Melanospora destruens</i> . . . . .	0	—	—	10	0	0.05	0.1	0.2
<i>M. zamiae</i> . . . . .	0.2	0.5	0.5	1.0	0.1	0.2	0.5	1.0
<i>Podospira curvula</i> . . . . .	0.2	—	0.5	0.5-1.0	—	—	—	—
<i>Philocopa</i> sp. . . . .	0.5	—	1.0	1.0-2.0	—	—	—	—
<i>Sordaria fimicola</i> . . . . .	0.1	0.5	0.5-1.0	1.0	—	—	—	—
<i>Chaetomium cochloides</i> . . . . .	—	—	—	—	0.2	0.2	0.3	0.5
<i>Pyronema confuens</i> . . . . .	0.2	—	0.5-1.0	—	—	—	—	—
<i>Collybia velutipes</i> . . . . .	1.0	—	1.0-2.0	2.0	—	—	—	—
<i>Coprinus ephemerus</i> . . . . .	1.0	—	2.0	5.0+	0.1	—	1.0	1.0-5.0
<i>Schizophyllum commune</i> . . . . .	—	—	3.0-5.0	—	0.8	2-2.5	—	2.0-3.0
<i>Hydnum coralloides</i> . . . . .	0.1-0.5	—	0.5-5.0	—	—	—	—	—
<i>Zygorhynchus moelleri</i> . . . . .	—	—	5.0+	—	—	—	—	—
<i>Rhizopus sexualis</i> . . . . .	0.5	—	0.5-2.0	2.0	0.1	0.5	0.5	1.0
<i>Phycomyces nitens</i> . . . . .	—	—	15.0+	—	—	—	—	—
<i>Basidiobolus ranarum</i> . . . . .	0.5	—	5.0-10.0	—	—	—	—	—
<i>Phytophthora cactorum</i> . . . . .	1.0	—	1.0-2.0	2.0	—	—	—	—
	0.1	—	0.1-0.5	0.5	—	—	—	—

<sup>1</sup> Data from Keyworth (l.c.).

5.0+

system of values was evolved to take into consideration both size and number of fructifications, and this gave consistent results.

A similar relation between concentrations of glucose and vitamin B<sub>1</sub> and the production of zygotes by *Zygorhynchus moelleri*, *Rhizopus sexualis*, and *Basidiobolus ranarum* and of oogonia by *Phytophthora cactorum* was demonstrated. Plates of medium A in which KNO<sub>3</sub> was replaced by asparagin were inoculated at a standard distance with the + and — strains of *Phycomyces nitens*. The number of conjugations along the line of junction of the two colonies was counted. With high concentrations of both glucose and vitamin B<sub>1</sub> the number of conjugations was so great that very few were able to develop into mature zygotes, some other nutrient such as asparagin being probably a limiting factor. Again the concentration of glucose optimal for fruiting increased with increasing concentration of vitamin B<sub>1</sub>.

All the results obtained are summarized in the Table, which gives the concentrations of glucose (gm. per 100 c.c. medium) optimal for fruiting at different concentrations of aneurin or of lentil concentrate. It was ascertained by the *Phycomyces* test (Schopfer and Jung, 1937; Hawker, 1939a) that 0.05 gm. dry wt. of lentil extract contained approximately 1  $\gamma$  vitamin B<sub>1</sub>. A plus sign after a figure indicates that no higher concentration of glucose was tried, and that while this highest amount gave the best results it was not necessarily the optimum value, which was probably higher still.

Thus in a wide range of fungi fruiting depends on a balance between the concentrations of carbohydrate (glucose) and of vitamin B<sub>1</sub>. In all the fungi examined, including *Rhizopus*, *Pyronema*, and *Chaetomium* which normally fruit freely in the absence of an external supply of growth substances, fruiting falls off if the concentration of sugar be increased sufficiently. The addition of vitamin B<sub>1</sub> raises both the optimal and maximal concentrations of glucose for fruiting. This relation between vitamin B<sub>1</sub> and glucose may afford a valuable clue to the interpretation of the part played by the former in the production of fruit bodies by fungi, and further work on these lines is being carried on in this laboratory.

#### SUMMARY

The effect of varying concentrations of glucose and of vitamin B<sub>1</sub> on the fruiting of a wide range of fungi resembles that already described for *Melanospora destruens*. With all fungi examined the concentration of glucose optimal for fruiting rose with increasing concentration of the vitamin.

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# The Relation of Yeast Multiplication to the Composition of Apple Juice

BY

HUGH DICKSON

With seven Figures in the Text

## I. INTRODUCTION

THE experiments on the fermentation of apple juice described below were carried out at the University of Bristol Research Station, Long Ashton, in the winter of 1938 and in 1939 prior to the outbreak of war. The experiments described are purely of an exploratory nature, as little work in the laboratory had previously been carried out on the physiological aspects of fermentation in this medium.

## 2. EXPERIMENTAL PROCEDURE

In the preparation of the juice apples were first milled, then pressed and pulp-filtered, and then filtered through a Seitz K filter film which produced a 'brilliant' juice practically free from yeast cells and other micro-organisms.

Three different yeasts were used in the experiments, namely two strains of *Saccharomyces ellipsoideus*, one used in cider production on the continent and known as strain 41, the other, strain A, taken from a local cider; the third was fresh commercial baker's yeast (*S. cerevisiae*).

Cell counts were made by means of a haemocytometer. The specific gravity determined with a standard hydrometer served as a convenient measure of the amount of sugar broken down, other substances removed by the yeast during fermentation having but a small effect on the gravity.

The yeast cultures in the juice were incubated in flasks at 26° C., and in most cases the medium was kept agitated and the yeast cells were prevented from settling by placing the flasks on a table kept in motion by means of an eccentric drive from a constant speed electric motor.

The figures for total nitrogen were obtained by the micro Kjeldahl method of Parnas and Wagner (1921) with slight modification. Details of the method are as follows: 2 ml. of filtered juice are measured into a Pyrex micro Kjeldahl flask followed by 1 ml. of hydrogen peroxide (90/100 vols.), 0.8 gm. of a potassium sulphate mixture containing copper sulphate and selenium and 2 ml. of pure concentrated sulphuric acid. The use of hydrogen peroxide greatly facilitates the digestion of carbonaceous material and considerably reduces frothing in the preliminary stages. Heat is applied gently

at first until the mixture shows signs of clearing and then strongly for a further 20 minutes. The contents of the flask are transferred to a Parnas-Wagner distillation apparatus together with 15 ml. of 30 per cent. caustic

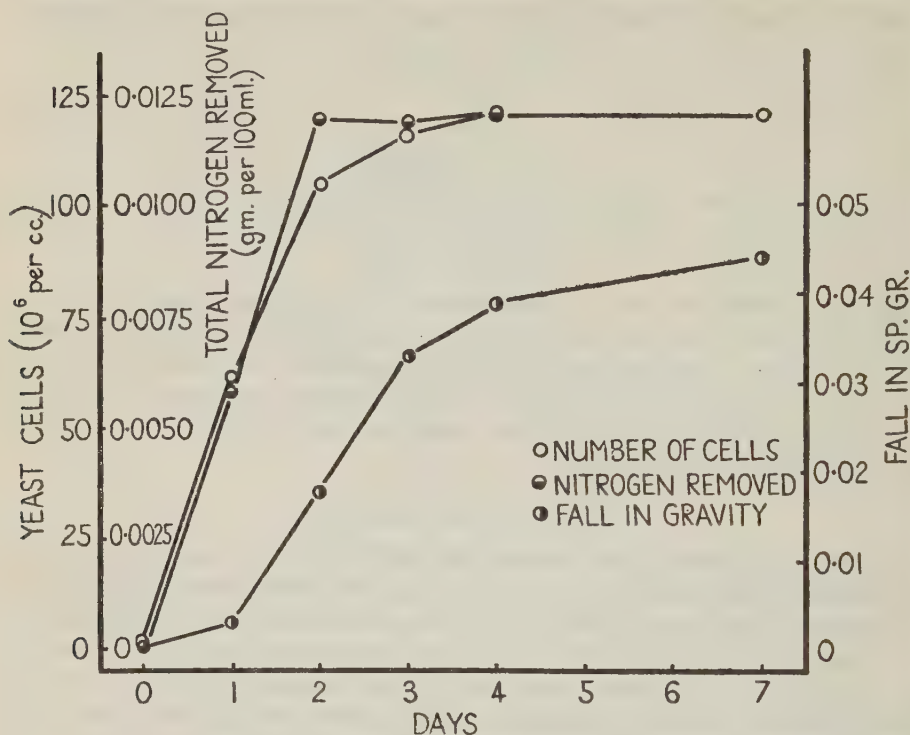


FIG. 1. Curves showing the increase in cell number, the fall in gravity, and the removal of nitrogen during fermentation.

soda containing 5 per cent. sodium thiosulphate. The ammonia produced is steam-distilled into N/70 hydrochloric acid for 4 minutes. Finally the latter is back-titrated with N/70 caustic soda using Tashiro's indicator (2 ml. of 1 per cent. aqueous methylene blue + 100 ml. of 0.04 per cent. methyl red in 50 per cent. alcohol).

### 3. DETAILS OF EXPERIMENTS AND EXPERIMENTAL RESULTS

*Experiment 1.* Fresh apple juice was 'seeded' with strain 41 at approximately  $1.5 \times 10^6$  cells per ml. and incubated. The original gravity was 1.046 and the total nitrogen content of the juice 0.0147 gm. per 100 ml.

Fig. 1 shows the fall in gravity, the increase in the number of yeast cells, and the amount of nitrogen removed (gm. per 100 ml.) during the growth of the yeast. It is seen that the number of cells increases rapidly at first and then more slowly, till from the fourth day it remains practically constant. Other experiments have shown, however, that the curve of increase in cell

number with time is in effect sigmoid, being at first approximately logarithmic, as has been found by other workers on different media (cf. Hopkins, 1927). That this has not been found in the present instance is due to the length of the interval between inoculation and the first count.

The curve of the fall in gravity with time is sigmoid and was still falling slowly on the seventh day, when it reached 1.002. The minimum usually attained with apple juice is 0.998, the fall below zero being due to the alcohol then present.

The nitrogen removed from the juice, i.e. that removed by the yeast in growth, gives a curve which follows very closely that of yeast counts. It reaches a maximum on the second day, when only 0.0027 per cent. is left in the juice. A final value of 0.0025–0.0020 per cent. is usually attained in a juice which has just been fermented to completion and in numerous tests no lower figure has ever been obtained. It appears, therefore, that on the total nitrogen content of a juice reaching a value of 0.002 per cent. the yeast is either unable to withdraw further nitrogen from the liquid or else a balance is established at this figure between the amount withdrawn by the yeast and that returned to the medium by autolysis of dead cells.

It is seen from this experiment that all available nitrogen in the juice is used up and yeast multiplication ceases for a fall in gravity of approximately 0.02.

*Experiment 2.* Apple juice was seeded with strain 41 at  $0.5 \times 10^6$  cells per ml. Fig. 2 shows the increase of cell number with time; curve *A* is for a fresh unfermented juice, curves *B*, *C*, and *D* in the same juice inoculated and fermented for 1, 2, and 3 days respectively, then Seitz K filtered and reinoculated. These curves consequently show the effect on yeast multiplication as the nutritive constituents of a juice are used up in fermentation, the most notable feature being the rapid falling off in the capacity of the juice to promote multiplication after only one day's fermentation, i.e. the difference between curves *A* and *B*.

*Experiment 3.* A juice produced from mixed culinary apples was inoculated with strain 41 and allowed to ferment till a gravity of 1.026 was reached. It was then filtered, reinoculated with a small quantity of strain 41 divided into five parts of 70 ml. each, and the following quantities of different salts added to the respective flasks: *A*, control; *B*,  $(\text{NH}_4)_2\text{SO}_4$  0.08 g.; *C*, Fe a trace; *D*,  $\text{KH}_2\text{PO}_4$  0.05 g.; *E*, glucose 2.0 g.

Fig. 3 shows the yeast count per day for 3 days in each flask. It is seen that of those tried nitrogen is the most important element in its effect on the rate of yeast multiplication, the addition of the other salts having little or no significant effect.

*Experiment 4.* A juice produced by normal methods from Bramley's Seedling apples was used. It had the abnormally low nitrogen content of 0.002 per cent., and the addition of a small amount of  $(\text{NH}_4)_2\text{SO}_4$  was necessary to enable fermentation to proceed normally. As only a small amount of this

salt was added, the final number of cells produced did not exceed  $70 \times 10^6$  per ml., which permitted accurate counts of cell numbers to be readily made.

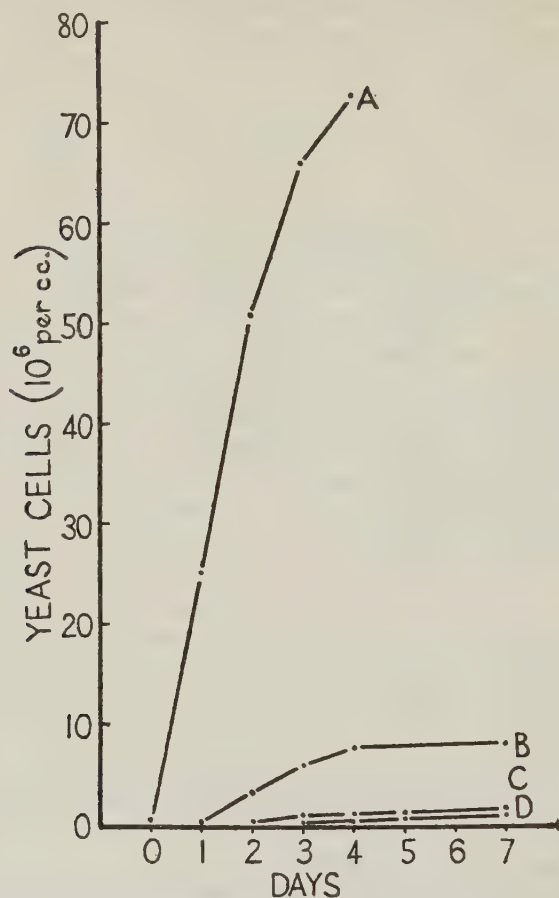


FIG. 2. Effect of fermentation of apple juice for successive days on the subsequent multiplication rate of yeast.

Yeast of strain A was used as inoculum and the flasks were agitated during fermentation.

The results are shown in Fig. 4, in which the log. of the cell number is plotted against time. The seven curves correspond with the seven seeding rates: 0.002, 0.023, 0.07, 0.22, 0.65, 3.25, and 13 million cells per ml. It is seen that the relative rates of increase in cell number decrease with rising seeding rates, and the final cell numbers tend to approximate, though with the exception of the two smallest seeding rates the greater the seeding rate the higher is the final cell count. The difference in the final cell numbers is probably due to differences in the amounts of nitrogen available, as a certain quantity is introduced in the yeast inoculum.



*Experiment 5.* A juice prepared from mixed culinary apples was divided into six flasks *A-F*, which were inoculated respectively with bakery yeast at the following seeding rates: 0.5, 0.1, 0.02, 0.004, 0.0008, 0.00016 gm. per litre. These inoculations gave 5, 1, 0.2, 0.04, 0.008, 0.0016 million cells per ml. respectively. The contents of each flask were filtered daily through a

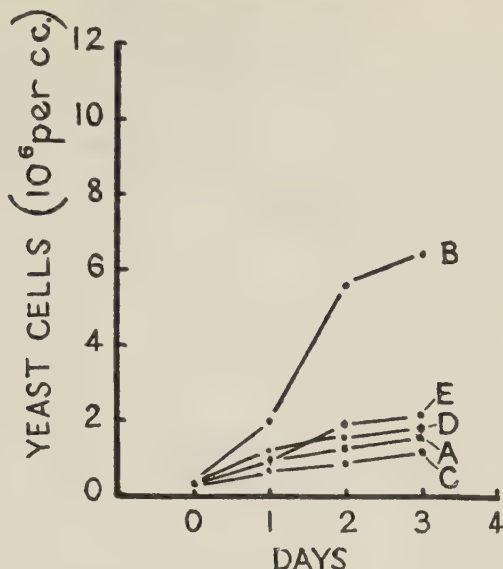


FIG. 3. The effect on the rate of cell division of adding different salts to a partly fermented juice.

Seitz K filter and immediately reinoculated at the same seeding rate as before. This method enabled one to study the effect on gravity and nitrogen content of a juice of different yeast concentrations reduced daily to a pre-determined level.

Fig. 5 shows the daily loss in gravity at the different seeding rates and Figs. 6 and 7 the rate of reduction of the total nitrogen in the juice with fall in gravity and with time. The C/N ratio remains approximately constant for the first part of the experiment in the case of each seeding rate, and then decreases. The curves of cultures *A* and *B* show an actual increase in the nitrogen content after reaching a minimum around 0.002 per cent. A similar result may occur at the other seeding rates, but readings were not continued for sufficiently long to show it. Such an increase in the total nitrogen has been observed in other experiments.

The amount of nitrogen removed from the juice for a given fall in gravity varies with the seeding rate. At all gravities it is greatest for seeding rates *B* and *C* and is progressively smaller for rates *D*, *E*, and *F* respectively. Rate *A* is approximately intermediate between *C* and *D*. The differences increase as the gravity decreases. I am indebted to Professor R. H. Hopkins

for a suggested explanation for this differential consumption of sugar and nitrogen with different yeast concentrations, namely that a lag phase ensues following the introduction of the yeast during which nitrogen may be assimilated without any accompanying growth and fermentation. The greater

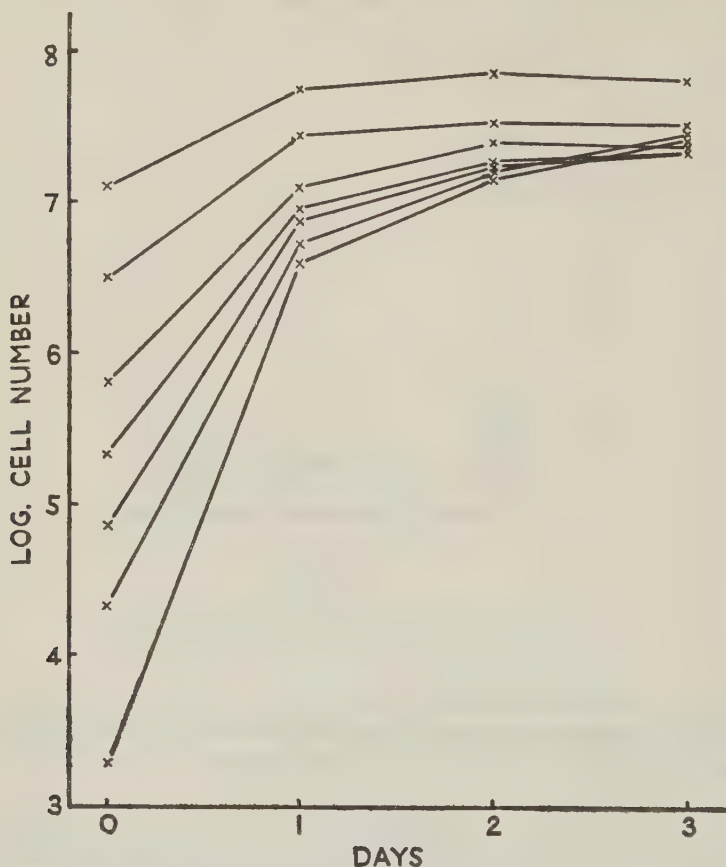


FIG. 4. Changes with time in the relative rates of increase in cell number.  
The curves represent different initial seeding rates.

the yeast inoculum the larger the effect produced, namely nitrogen removed without the equivalent consumption of carbohydrate. This suggestion is supported by an experiment in which it was found that on filtering and re-inoculating a culture every second hour for 6 hours more nitrogen was removed for a given fall in gravity than was the case when one inoculation at the same seeding rate was used and the experiment continued for 24 hours. On the assumption that the yeast on being introduced into the apple juice does not contain sufficient nitrogen to begin division and that a certain quantity has to be assimilated by each cell before growth can commence on

an appreciable scale (thus accounting for the lag phase) an explanation is readily forthcoming for the existence of a maximum value for the seeding

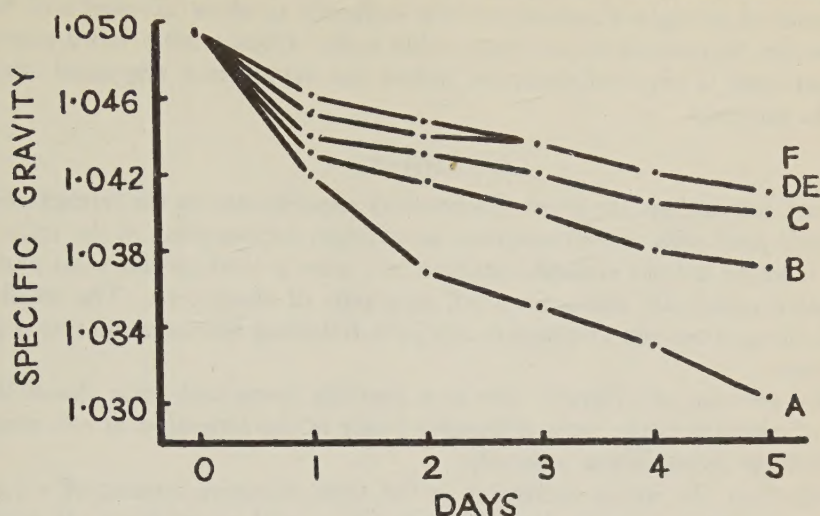
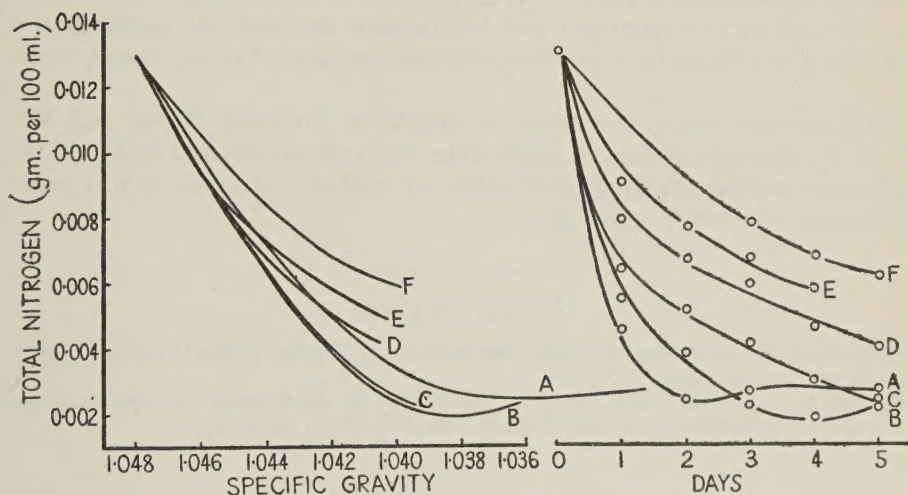


FIG. 5. Change of gravity with time as a result of six different seeding rates; A highest, F lowest.



FIGS. 6 and 7. Curves showing relation of reduction of nitrogen with fall in specific gravity (Fig. 6) and with time (Fig. 7).

rate such as has been found in the experiment described. Analysis shows that for every cell introduced into the medium on seeding the following relative amounts of nitrogen have been absorbed from the juice after 24



hours' incubation: *A*, 17; *B*, 75; *C*, 330; *D*, 1250; *E*, 4875 units. These differences are large and it may well be that at the higher seeding rates the amounts of nitrogen absorbed are not sufficient to allow division and fermentation to proceed on an appreciable scale. Considerably more experimental work is required, however, before the explanation suggested above can be accepted.

#### 4. SUMMARY

Brief descriptions are given of laboratory experiments on the fermentation of apple juice with special reference to nitrogen consumption in the juice.

It is found that all available nitrogen in a juice is used up and yeast multiplication practically ceases for a fall in gravity of about 0.02. The smallest total nitrogen content obtained in any juice following fermentation was 0.002 per cent.

The addition of different salts to a partially fermented juice shows that loss of nitrogen is the most important factor in the reduction of cell multiplication as fermentation proceeds.

Following the initial reduction in the total nitrogen content of a juice a small final increase has been recorded in several experiments; it occurs when fermentation is nearing completion, i.e. when all the sugar available has been used up.

The amount of nitrogen extracted from a juice for a given fall in gravity varies with the seeding rate. It was found that as the seeding rate increased it increased up to a maximum and then began to decrease. An explanation is advanced to account for this differential consumption of nitrogen and sugar.

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